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EFFECTS OF BOTULINUM TOXIN B ON REFRACTORY DETRUSOR OVERACTIVITY: A RANDOMISED, DOUBLE- BLIND, PLACEBO CONTROLLED, CROSS OVER TRIAL.

THESIS FOR MD

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DECLARATION

The work contained in this thesis is my own. None of the data is a part of any other thesis. The studies presented here were approved by the ethics committees of the individual hospitals. All patients gave informed consent prior to their involvement in the study.

Maneesh Ghei

ABSTRACT

INTRODUCTION

Open, observational studies of intradetrusor injections of Botulinum toxin for detrusor overactivity have reported beneficial effects. This thesis reports the testing of the efficacy and safety of Botulinum toxin B (BTX-B) for treatment of the overactive bladder in a randomised, double-blind, placebo controlled cross-over trial.

METHOD

20 patients, aged 18-80 years, with detrusor overactivity, and incompletely responsive to oral antimuscarinic agents, participated. They were injected with either placebo (20 mls normal saline) or botulinum toxin B (5000 IU diluted up to 20 mls) into the detrusor in a day case setting. After six weeks, the treatments were crossed over without washout. The primary outcome was the paired difference in change in the average voided volumes. Paired differences urinary frequency, incontinence episodes and in the quality of life (QOL), measured by the King's Health Questionnaire (KHQ), were the secondary outcome measures.

On the occasion of each injection two biopsies were taken from the detrusor. These were processed and scrutinised for evidence of inflammatory responses to the injection.

RESULTS

The Wilcoxon Signed Ranks Test was used to test the paired difference in change between treatment phases. Statistically significant paired differences in the change in average voided volume, urinary frequency and episodes of incontinence between active treatment and placebo (Av Void Vol: 95% CI diff 16, 122; Z = -2.5; p=0.012 / Weekly Freq: 95% CI –21, -1; Z = -2.1, p=0.033 / Weekly incont: 95% CI –26, -7; Z=-3.3; p=0.001) along with significant paired differences in five domains of the KHQ were observed.

CONCLUSIONS

This double-blind, placebo-controlled, cross-over study provides evidence of efficacy of Botulinum Toxin B in the treatment of the overactive bladder. Autonomic side effects were observed in four of the patients. The short duration of action will presumably limit the use to patients who have experienced tachyphylaxis with (Botulinum toxin A) BTX-A.

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I dedicate this thesis to my father, Ram, and my mother, Sudha. I would have never reached this far without their unfailing love and total dedication. 'I can never thank you enough.....'

CHAPTER - 1

Chapter 1 INTRODUCTION

1.1 HISTORY

Botulinum toxin has long been recognised as an extremely potent, naturally occurring poison [1]. It is a neurotoxin produced by the bacterium *Clostridium botulinum*. Christian Andreas Justinus Kerner, a German physician and poet, was the first to publish an account of botulism as a food-borne disease in spoiled pork sausages [2]. This substance, Kerner suggested, might be used in the future as a therapeutic agent in patients with involuntary movements as a result of neurological disease. However, it would be 160 years before the first therapeutic use of BTX was reported in humans. The actual bacterium was isolated by Ermengem after an outbreak of sausage-related poisoning in 1894 in the small village of Ellezelles, in Holland [3]. In 1944, military bacteriologists and physicians on both sides of the Atlantic were assigned to study and purify BTX, leading to the production of a crystalline form of type A BTX (BTX-A), one of the more common forms in human outbreaks [4]. In 1968, Schantz and Scott collaborated in developing a therapeutic formulation of BTX-A and used it successfully in correcting strabismus in experimental monkeys [5]. Of the seven neurotoxic serotypes (A-G), type A and type B have been introduced into clinical practice [6].

1.2 STRUCTURE

Although the seven neurotoxins are antigenically distinct, they possess similar molecular weights and have a common subunit structure [7]. The complete amino acid sequences for various toxins are becoming known [8-17].

The botulinum toxin molecule is synthesised as a single chain and then cleaved and spliced to form the dichain molecule with a disulfide bridge. In the single chain form the molecules have a mass of 150 kD and relatively little potency as neuromuscular blocking agents.

Neurotoxic activation requires a two step modification in the tertiary structure of the protein. In the first step, as already stated, the parent chain is cleaved and spliced. The result is a heavy chain (approx. 100kDa) tethered by a disulfide bond to a light chain (approx 50 kDa) that is associated with one atom of zinc [18].

For most forms of the toxin this step is accomplished by the bacterium itself before it is released.

The light chain amino acids (1-448) act as a zinc endopeptidase, with proteolytic activity concentrated at the N-terminal end.

The heavy chain (amino acids 449-1280) provides cholinergic specificity and promotes light chain translocation at the end of the endosomal membrane. [19]

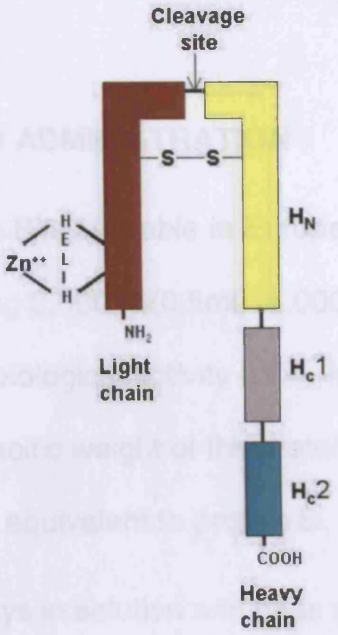

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Figure 1.1

Structure of Botulinum toxin showing the light chain and the heavy chain linked by a disulfide bond

1.3 FORMULATION AND ADMINISTRATION

Botulinum toxin (Btx) type B is available in Europe as Neurobloc™ and is supplied in vials containing 2,500 IU(0.5ml), 5,000 IU (1.0ml) or 10,000 IU(2.0ml). Units refer to biological activity (LD_{50} in the Swiss Webster mouse potency assay), not a specific weight of the protein. It should be noted that one IU of protein A is not equivalent to protein B.

Botulinum toxin B is always in solution and does not require lyophilization and so it retains almost 100% of its specific activity [20, 21].

Botulinum Toxin type A is stored unconstituted in the freezer (<= -5° C), however type B can be stored refrigerated (2-8 ° C) for upto 36 months and at room temperature for at least 9 months without losing stability or potency. The relative stabilities of the two toxins may reflect, in part, the slightly acidic formulation of type B at pH 5.6.

Use of this pH confers extended stability for type B product in solution. At more neutral or slightly basic pH values, botulinum toxin complexes completely dissociate, markedly affecting stability and potency.

Studies have shown that type B diluted upto six fold with either preserved or non preserved saline, remain stable and retain potency for at least 24hrs at room temperature[22].

1.4. MECHANISM OF ACTION

1.4.1 PHYSIOLOGY OF SMOOTH MUSCLE CONTRACTION

Since the effects of BTX are secondary to inhibition of acetylcholine release, it is important to discuss this neurotransmitter and the various receptors it acts on, in further detail.

Acetylcholine (Ach) was one of the first neurotransmitters to be discovered, (originally called 'vagusschtuff' because it was found to be the substance released by stimulation of the vagus nerve that altered heart muscle contractions).

1.4.2 SYNTHESIS OF ACETYLCHOLINE

Synthesis of acetylcholine is facilitated by the enzyme, choline acetyltransferase (CAT). This enzyme combines choline with acetate derived from acetyl coenzyme A (CoA). Choline is taken up into cholinergic nerves by a high affinity transport process (sodium-choline cotransport) that is indirectly coupled to the energy stored by the Na/K pump ATPase. This transporter process is inhibited by hemicholinium-3 (HC-3). HC-3 has no immediate effect on neurotransmission, but can cause cholinergic nerve fibres eventually to run out of transmitter. In the presence of HC-3, the more rapidly cholinergic fibres are stimulated, the more rapidly they run out of ACh.[23]

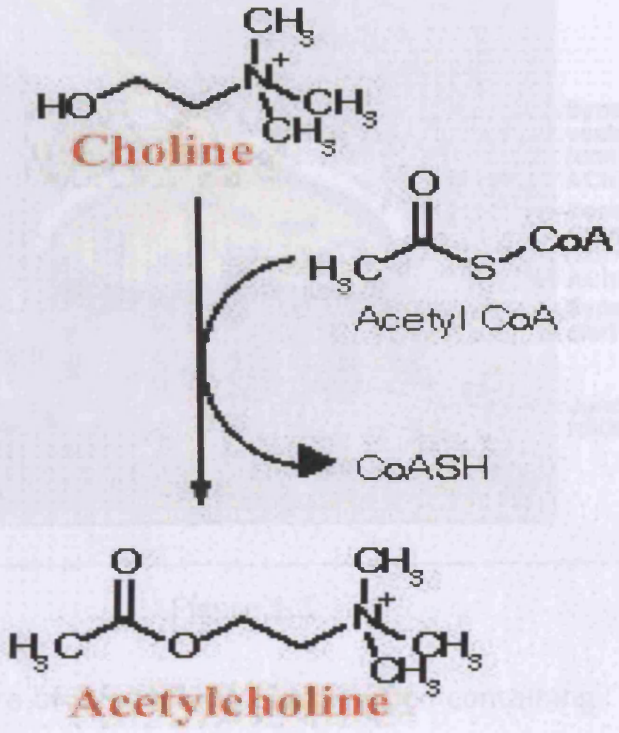


Figure 1.2

Chemical structure of acetyl choline

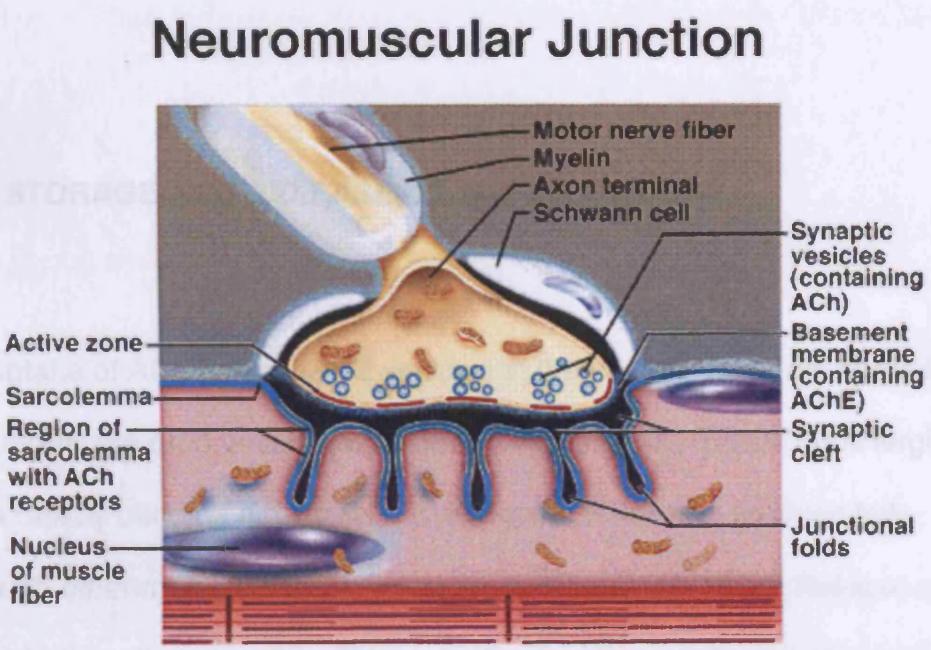


Figure 1.3

Structure of a neuromuscular junction containing acetyl choline vesicles

1.4.3 STORAGE AND RELEASE OF ACETYLCHOLINE

The uptake of ACh into synaptic vesicles in the cholinergic nerve fibres is inhibited by the drug vesamicol. In the presence of vesamicol, cholinergic fibers rapidly become depleted in stored ACh. Neurotransmission fails although other metabolic functions of the fibres are still intact. Release of acetylcholine, like synaptic release at other junctions, is based on quantal release of vesicles containing preformed neurotransmitter molecules. Vesicular release depends on depolarization of the nerve terminal and the influx of calcium ions. In ways not yet understood in detail, the influx of calcium promotes simultaneous exocytosis of many vesicles. At the motor end-plate of the neuromuscular junction this results in a massive release of ACh (hundreds of vesicles and thousands of ACh molecules per vesicle) and substantial depolarization of end-plate potential that normally results in contraction of the muscle cell. The effect of background quantal release of ACh-containing vesicles can be observed as miniature end plate potentials (mepps). The release of ACh at various cholinergic junctions can be blocked by certain toxins, most notably those produced by Clostridium species. Botulinum toxin , from Clostridium botulinum binds to cholinergic nerve terminals and is internalized. Once internalized it acts on the vesicle release process and prevents exocytosis. All junctional release of ACh is inhibited by

such toxins. In patients poisoned by Clostridium botulinum the immediate clinical problem is flaccid paralysis and respiratory failure.[23]

1.4.4 ACTIONS OF ACETYLCHOLINE AND RECEPTORS

Acetylcholine (ACh) has diverse actions on a number of cell types mediated by two major classes of receptors. *Nicotinic receptors* are ligand-gated ion channels. Muscarinic receptors are part of the transmembrane, G protein coupled receptor family.

There are two major subtypes of nicotinic receptors; those found in the neuromuscular junction of skeletal muscle (nicotinic muscle, Nm) and those found in autonomic ganglia and other parts of the nervous system (nicotinic neuronal, Nn). When ACh or other agonists occupy the receptor site on the external surface of the cell membrane there is a conformational change in the ion channel and an increase in conductance to the ion(s) for which that channel is selective. Thus, when Nm receptors are activated, there is an influx of cations through the ion channel and depolarization of the motor end plate. In short, nicotinic receptors directly transduce the ACh external messenger into an action on the cell. [23]

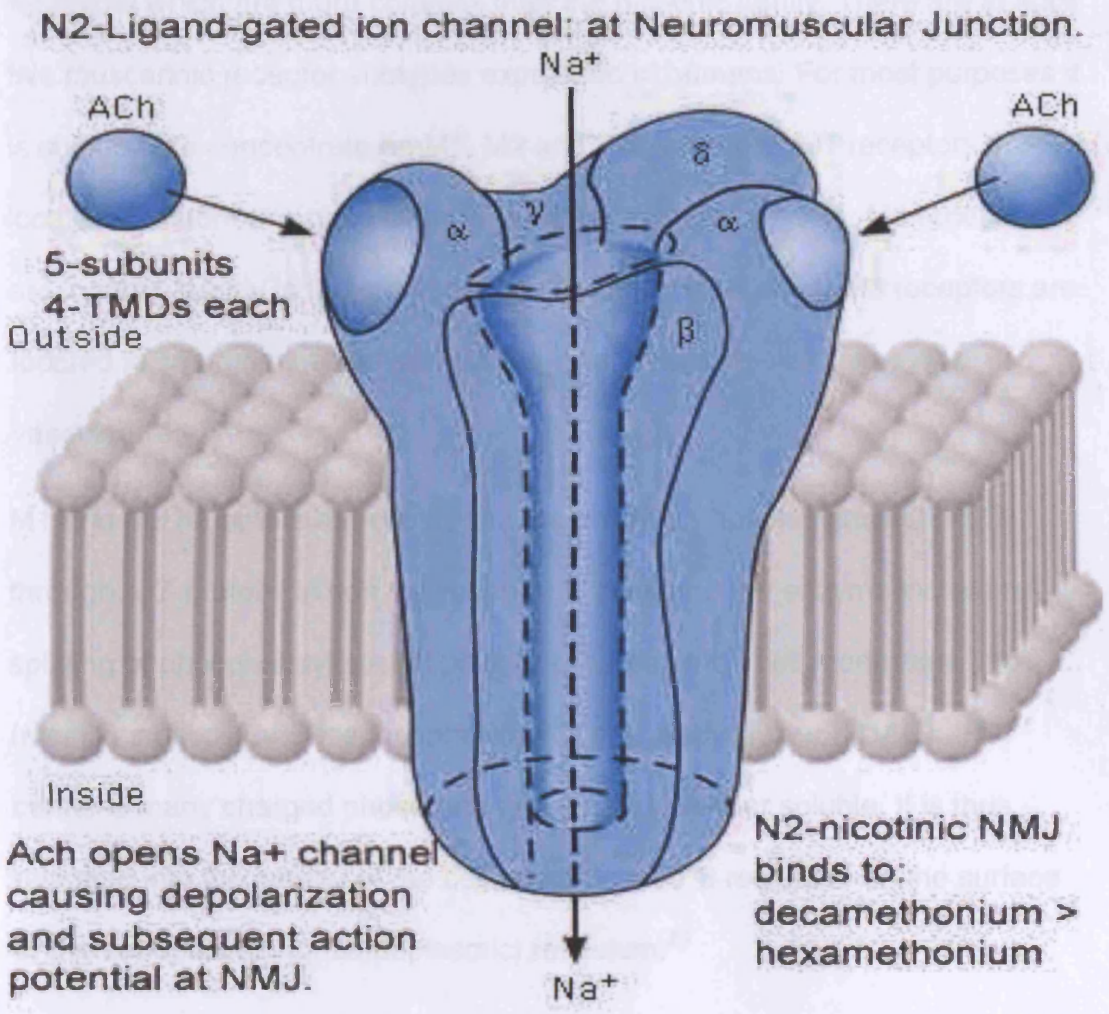


Figure 1.4
N2 ligated- gated ion channel at the Neuromuscular junction

Transduction of the ACh message is more complex in the muscarinic family of receptors which are more complex than the nicotinic family. There are at least five muscarinic receptor subtypes expressed in humans. For most purposes it is sufficient to concentrate on M1, M2 and M3 receptors. M1 receptors are located in autonomic ganglia and the central nervous system. M2 receptors are located mainly in the supraventricular parts of the heart. M3 receptors are located in smooth muscles and glands, and on endothelial cells in the vasculature.

M1 and M3 receptors are coupled to the enzyme phospholipase C (PLC) through a G protein. When the receptor is activated the enzyme increases splitting of phosphatidylinositol polyphosphates of the cell membrane into (mainly) inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 contains many charged phosphate groups and is water soluble. It is thus released into the interior of the cell and acts on IP3 receptors on the surface of the endoplasmic (or sarcoplasmic) reticulum.[23]

IP3 receptors increase the release of Ca^{++} from the ER and increased cytosolic Ca^{++} is thus part of the intracellular message from ACh at the surface membrane. In the example illustrated here, an M3 receptor on a smooth muscle cell promotes smooth muscle contraction by promoting increased cytosolic free Ca^{++} ion.

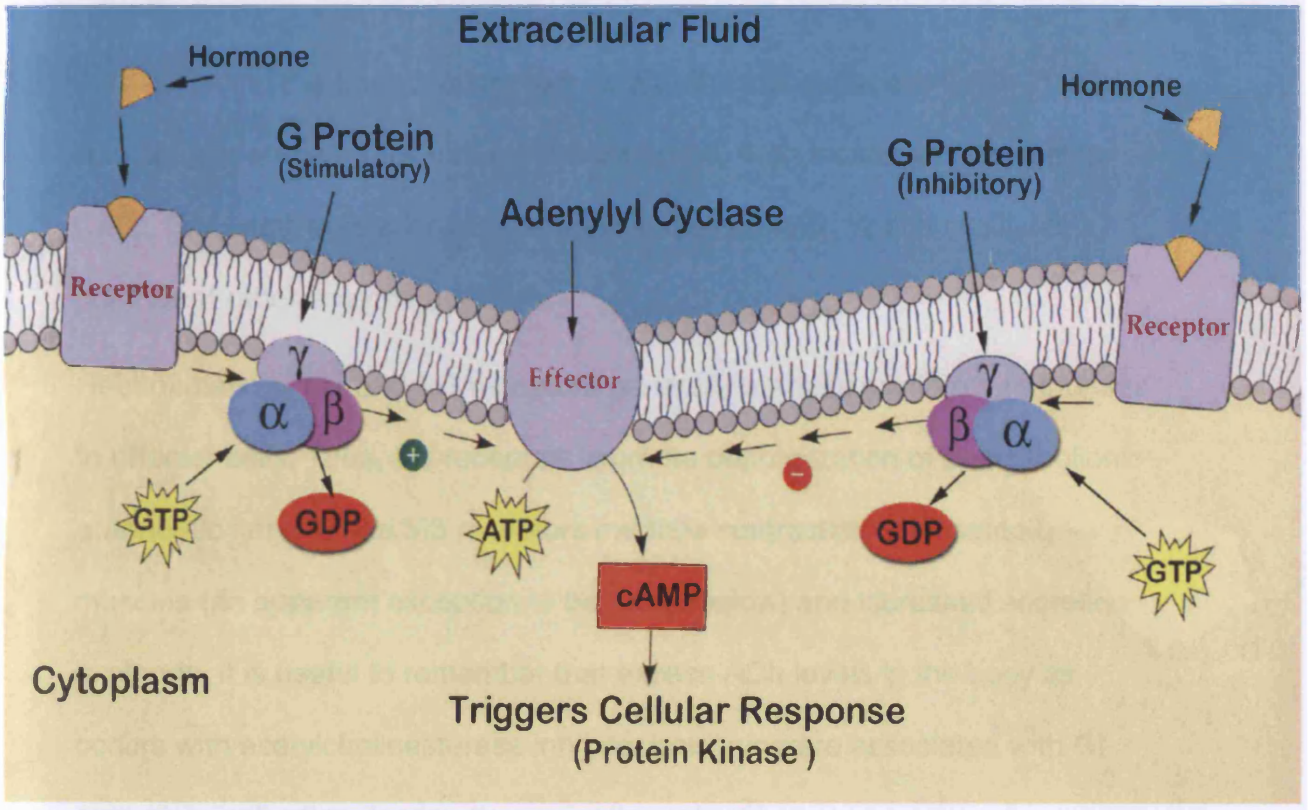


Figure 1.5
Muscarinic receptors
with G protein

Another link in the transduction from ACh at the cell surface is DAG. This is lipid soluble and remains in the cell membrane. With increased intracellular Ca^{++} , DAG activates a kinase, protein kinase C (PKC). Which regulates a number of other enzyme activities.

Recapitulating, M1 and M3 receptors generally mediate excitatory responses in effector cells. Thus, M1 receptors promote depolarization of postganglionic autonomic nerves, and M3 receptors mediate contraction of all smooth muscles (an apparent exception to be noted below) and increased secretion in glands. It is useful to remember that excess ACh levels in the body as occurs with acetylcholinesterase inhibitor poisoning are associated with GI cramping, salivation, lacrimation, urination, etc. [23]

1.5 DIFFERENT TARGET SITES FOR DIFFERENT BOTULINUM TOXINS

The botulinum toxins are synthesised as single chain polypeptides with a molecular mass of approximately 150kDa. In this form, the toxin molecules have little potency as neuromuscular blocking agents.

Neurotoxic activation requires a two stage modification in the tertiary structure of the protein [24]. In the first step, the parent chain is cleaved and spliced. The result is a heavy chain (approx 100 kDa) tethered by a disulfide bond to a light chain (approx 50 kDa) that is associated with 1 atom of zinc [25]. For most forms of botulinum toxin, including type A, this cleavage is accomplished by the bacterium itself before the toxin is released and enters the axon terminal. The second activating step, disulfide reduction, occurs only after internalisation by the target cell.

There are three processes involved in toxin mediated paralysis;

(1) Internalisation, (2) Disulfide reduction and translocation, and (3) Inhibition of neurotransmitter release.

The toxin must enter the nerve ending to exert its effect [26]. Binding of toxin to both peripheral and central nerves is selective and saturable. Pharmacologic and morphologic data suggest that the internalisation is via a receptor

mediated endocytotic / lysosomal vesicle pathway [27-30] The process is independent of Ca^{2+} concentration, is partially dependent on nerve stimulation, and requires energy [31]. In experimental systems, internalisation is hastened in an acid medium and slowed by cooling.

In vitro studies with synaptosomal preparations suggest heterogeneity of receptor sites, with both high and low affinity ligands and some specificity for toxin type [32, 33]. The C terminal half of the heavy chain determines cholinergic-nerve specificity and is responsible for binding, while the light chain is the intracellular toxic moiety. If the disulfide bond that links the two chains is broken before the toxin is internalised by the cell, the light chain cannot enter and there is complete loss of toxicity. After internalisation, the disulfide bond is cleaved by an unknown mechanism, and the end terminal half of the heavy chain promotes penetration and translocation of the light chain across the endosomal membrane. This ultimately disrupts the secretory pathway for acetylcholine [34]. Botulinum toxin specifically targets acetylcholine release, rather than synthesis or storage [34, 35]. Nerve endings which are poisoned with the toxin can still be induced to release the normal quanta of acetylcholine, although non physiologic techniques must be used.

Like tetanus toxin, Botulinum toxin is a zinc endopeptidase. The proteolytic activity is located in the N-terminal 50 kDa light chain. The binding of the zinc ion to the light chain is reversible and involves coordination with histidines, as is characteristic of zinc endopeptidases [36]. Acetylcholine release is inhibited

when the endopeptidase cleaves one or more neuronal proteins of the vesicle transport pathway.

The ATP dependent translocation of an intact vesicle from the cytosol to the plasma membrane requires a suite of specific proteins. Cleavage of any of these proteins interferes with the proper binding and fusion of a vesicle to the plasma membrane, and therefore impedes exocytosis mediated neurotransmitter release.

The intracellular traffic patterns responsible for this translocation have become clearer during the past several years. Vesicle docking on the inner membrane surface involves the formation of a complex including cytoplasmic proteins (gamma- SNAP, alpha-SNAP, NSF, SNAP-25), vesicle proteins (VAMP/Synaptobrevin), and proteins on the target membrane (Syntaxin). The clostridial neurotoxins are now known to target some of these proteins. [37]

Schiavo and Dasgupta, showed the intracellular substrate for both Botulinum toxin-B and tetanus toxin to be the vesicle protein, (VAMP/Synaptobrevin-2) [38]. The botulinum toxin-B light chain activity is specific for VAMP/Synaptobrevin-2 (VAMP/SYB-2), as shown in the rat where this isoform is targeted while VAMP/Syanptobrevin-1 is spared. This proteolysis inhibits the release of neurotransmitter. Botulinum toxin-D and F also cleave VAMP/Synaptobrevin-2, but at a different site than BTX-B [39]. BTX-A and BTX-

E cleave another translocation protein, SNAP 25, and BTX-C acts by cleaving syntaxin [40](figure 1.6)

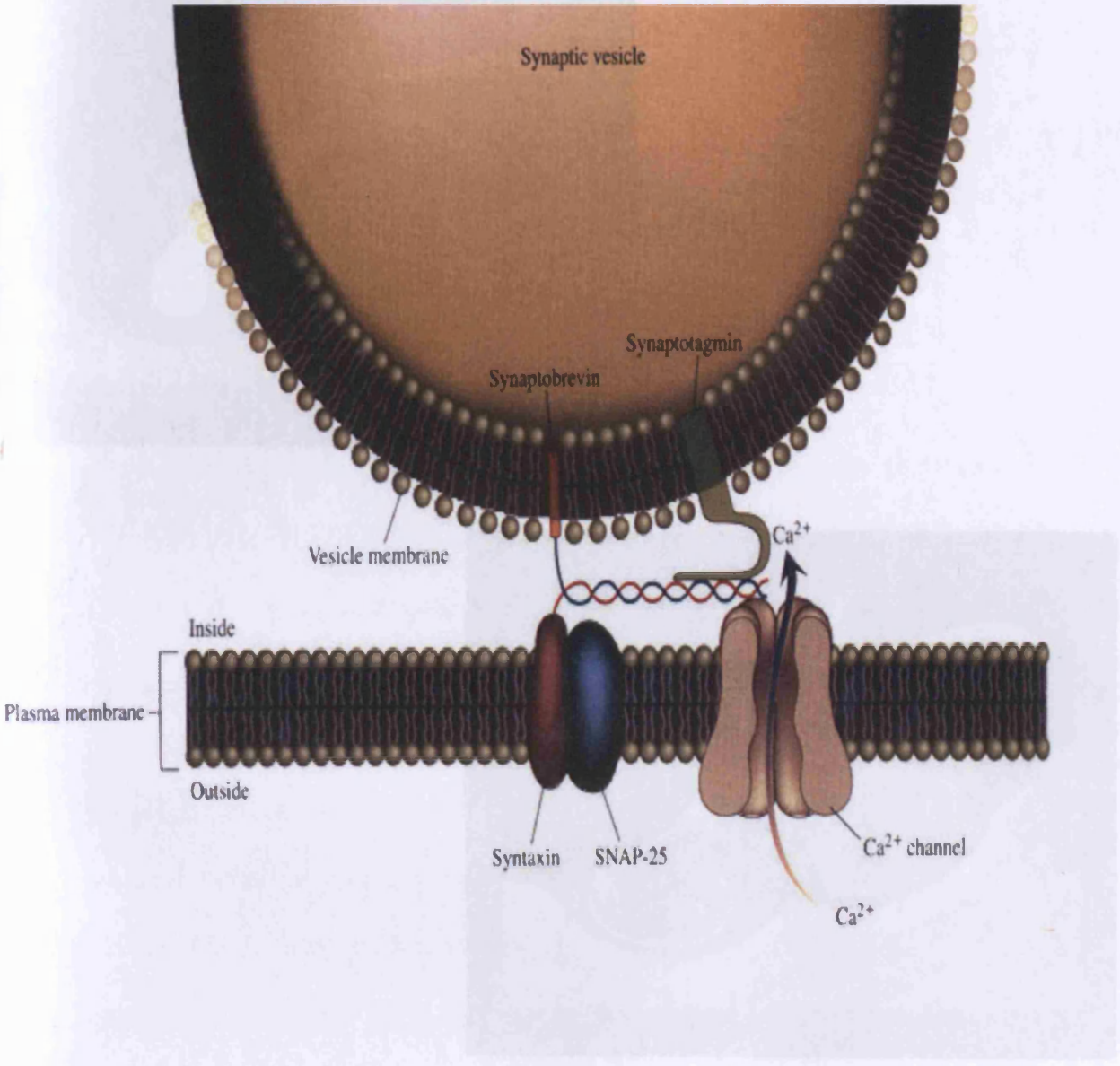


Figure 1.6 Targets for proteolysis by Botulinum toxins in the SNARE complex. Different botulinum toxins mediate paralysis by cleaving different SNARE proteins, each at distinct site. Serotypes A, C_1 and E cleave the SNAP -25 molecule. Serotypes B, D, F and G cleave synaptobrevin.

SPECIAL NOTE

THIS ITEM IS BOUND IN SUCH A MANNER AND WHILE EVERY EFFORT HAS BEEN MADE TO REPRODUCE THE CENTRES, FORCE WOULD RESULT IN DAMAGE

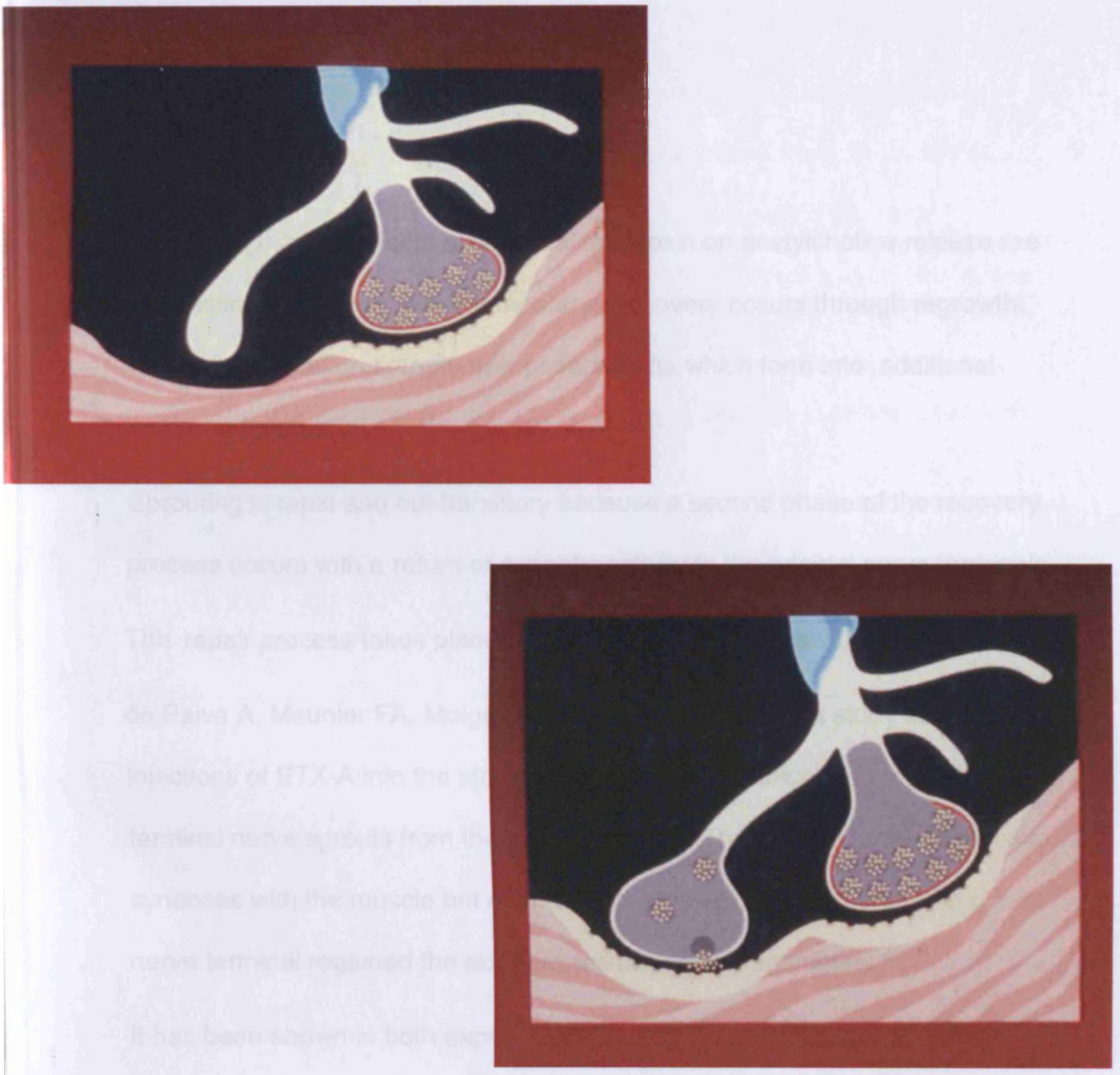


Figure 1.7
When acetylcholine is no longer able to be released, the nerve impulses no longer make the muscles contract. Over time, the nerve creates new endings in a process called sprouting. These new nerve terminals establish contact with the muscle and the effect of Botulinum toxin *wears* off.

1.6 RECOVERY

It is known that the effects of the botulinum toxin on acetylcholine release are long lasting but nevertheless temporary. Recovery occurs through regrowth. The end of the axon sprouts new pseudopodia which form into additional motor end plates.

Sprouting is rapid and but transitory because a second phase of the recovery process occurs with a return of synaptic activity to the original nerve terminals.

This repair process takes place over about 3 to 6 months.

de Paiva A, Meunier FA, Molgo J, et al. 1999, showed in a study involving 31 Injections of BTX-A into the sternomastoid muscle of mice, the formation of terminal nerve sprouts from the parent terminal. The sprouts formed functional synapses with the muscle but eventually regressed at a time when the parent nerve terminal regained the ability to release neurotransmitters [41].

It has been shown in both experimental animals and humans, long-term exposure to toxin causes reversible denervation atrophy [42-45]. Alderson examined muscle-nerve interactions in the orbicularis oculi for treatment of blepharospasm [46]. They found noncollateral sprouting that often ran parallel to muscle fibres and originated from three sources:

1. The nodes of ranvier of myelinated parent pre-terminal axons.
2. the unmyelinated terminal axon immediately proximal to the end plate; and

3. the ultraterminal axonal arborisation over the end plate

In addition some nerve plates appear segmented, with each segment innervated by an axonal process from the same motor axon. In some cases, more than one end plate was present on a single muscle fibre, each innervated by axonal processes from different preterminal axons. No necrosis or inflammation has been observed in human studies[47].

It remains to be seen whether similar processes occur in autonomic nerves innervating the lower urinary tract.

1.7 IMMUNOGENECITY

As is the case with many medications, some patients fail to respond to botulinum toxin when it is first administered. This can be termed a primary non-response. Tachyphylaxis can also develop giving a secondary response failure and this is known to have, at least in part, an immunological origin. Primary non-response has yet to be characterised so an antibody mediated reaction has not yet been excluded.

Botulinum toxins are peptides and will therefore exhibit immunogenic properties which are significant. Resistance has been attributed to antibodies directed against the toxin. Repeated use, particularly in high dose, has been associated with resistance arising from the development of neutralising antibodies [48-50]. Resistance is characterised by the absence of muscle weakness after injection.

Botulinum toxin preparations used therapeutically contain several antigenic proteins, in addition to the toxin itself. By no means have all of the antibodies formed had the ability to neutralise the therapeutic activity of the toxin. An antibody may be directed against an epitope that is found in a non-essential domain. Several laboratory generated monoclonal antibodies have been shown to react with botulinum toxin molecules without neutralising activity.

The botulinum toxin serotypes, were characterised by their antigenic properties. Botulinum toxins are large molecules with significant homology amongst serotypes. Although several antibodies that cross react with different types of botulinum toxin have been generated in the laboratory, there have been no reports of neutralising activity among antibodies that cross-react with different botulinum toxins serotypes. Botulinum toxins A and B have significant homology and exhibit a set of cross-reacting antibodies. To date the cross-reacting antibodies have not been found to show neutralising properties. [51-56]. Nevertheless it would be unwise to assume that such an occurrence were impossible.

Brin et al enrolled 77 patients suffering from cervical dystonia who had developed resistance to Botulinum toxin type-A into a 16-week, single treatment, double-blind, placebo controlled trial of Botulinum toxin type B. Improvements in severity of disability and pain were documented in the Botulinum toxin type B-treated patients [57].

Brashear et al enrolled 109 patients with cervical dystonia, who had manifest primary resistance to botulinum toxin type A, in a 16-week, randomised, multi center, single-treatment, double-blind, placebo controlled trial of Botulinum toxin type B. Placebo or 5,000 U or 10,000 U of the toxin were used. Significant improvements in pain, disability, and severity of cervical dystonia occurred for patients who were treated with botulinum toxin B as opposed to the placebo. The authors concluded that botulinum toxin B was safe and

efficacious at 5,000 U and 10,000 U for the management of patients with type A–responsive dystonia [58].

It has been shown that more frequent injections and higher doses per booster session contributed to a greater risk for antitoxin antibody formation [59-61].

However, more recently, Jankovic et al in their study on patients with cervical dystonias demonstrated a very low immune response and antibody formation using a newer formulation of Botulinum toxin A [62]. This contained less of a plethora of different proteins.

1.8 THERAPEUTIC USES OF BOTULINUM TOXIN

1.8.1 BOTULINUM TOXIN AND MOVEMENT DISORDERS

In 1968 Edward Schantz, an American army officer, working with the type A toxin, was approached by Alan Scott, an ophthalmic surgeon. Schantz and Scott collaborated in developing BTX-A and used it successfully in correcting strabismus in experimental monkeys. Progress in humans was slowed by a treaty in 1972 banning research into biological weapons, and it was not until some years later that USA Food and Drug Administration approval was given for civilian research on human volunteers. The first publication on the use of BTX-A in humans was in 1980 [5].

Over the last two decades, the use of BTX-A has become widespread in the treatment of various disorders of muscle overactivity. It is now licensed in the UK to treat strabismus, spasmodic torticollis, blepharospasm, hemifacial spasm and paediatric cerebral palsy and spasticity. However, numerous other unlicensed applications have been reported, including oesophageal spasm, sphincter of Oddi dysfunction in the gastrointestinal tract, cosmetic facial wrinkle reduction, palmar hyperhydrosis and hypersalivation, to name but a few [63]. The use of the agent in cosmetic treatments has attracted considerable public interest.

1.8.2 BOTULINUM TOXIN AND LUTS

1.8.2.1 Botulinum toxin and Neurogenic detrusor overactivity (NDO) and Idiopathic Detrusor overactivity (IDO)

In 2000 Schurch et al published their results with intradetrusor injections in 31 neurologically impaired patients. These patients with traumatic spinal cord injury emptied the bladder by intermittent self-catheterization. These patients had severe detrusor hyperreflexia and incontinence despite a high dose of anticholinergic medication. Under cystoscopic control a total of 200 to 300 units of botulinum-A toxin were injected into the detrusor muscle at 20 to 30 sites (10 units per ml. per site), sparing the trigone. Pre-treatment evaluation included a clinical examination and complete urodynamic investigation They reported a significant improvement in all urodynamic variables and post void residual volumes. Improvement in urodynamic variables and incontinence was present in all patients re-evaluated at 16 and 36 weeks [64].

In 2003, the same author reiterated that the preliminary results of intradetrusor injections of Botulinum toxin A in neurogenic detrusor overactivity were promising, however the optimum dose of the toxin required for this indication was not yet known. It was concluded that botulinum toxin injection into either the external urethral sphincter or the detrusor offered promise for many different urological dysfunctions. Nevertheless evidence from proper

controlled trials was required to justify the role of botulinum-A toxin injections in the fields of urology and neurourology [65].

Ten European medical centres published the results from 231 patients with neurogenic detrusor overactivity who were treated with BTX-A. 300 units of Botulinum toxin A were injected cystoscopically into the detrusor muscle at 30 different locations, while sparing the trigone. Urinary continence status, concomitant anticholinergic medication use, and patient satisfaction were recorded. Key urodynamic variables at baseline and at the first and second urodynamic follow-up examinations were analysed. This retrospective European multicentre study, presented an extensive experience with BTX-A injections into the detrusor muscle to treat neurogenic incontinence due to detrusor overactivity. The data supported safety and efficacy but being non-comparative and unblinded interpretation must be extremely guarded [66].

A team of Italian researchers, in March 2004, published a study of seventy-five patients with spinal cord injury and refractory detrusor overactivity. Of these, 35 patients received repeated intravesical instillations of resiniferatoxin (RTX) dissolved in normal saline; 40 patients received repeated injections of 300 units Botulinum toxin A diluted in 30 ml normal saline. Clinical assessment and urodynamics were performed at baseline and 6, 12 and 24 months after treatment. This study was neither blinded nor randomised. It was observed that intravesical RTX and botulinum-A toxin injections into the detrusor muscle was associated with improvement in symptoms and increase

in bladder capacity on urodynamic testing. Patients receiving Botulinum-A toxin injection showed greater clinical benefit and urodynamic change than intravesical RTX [67, 68]. The trial design pleads caution. It should be noted that when RTX was submitted to double-blind, randomised, placebo-controlled scrutiny, efficacy was not detected.

In May 2004, Kuo et al investigated the urodynamic changes after detrusor injection of botulinum A toxin in 30 patients with detrusor overactivity refractory to treatment with anticholinergic agents. This was an open non-comparative, observational study. 200 U of Botulinum toxin A was injected at 40 sites in the detrusor. Urodynamic variables and symptom scores were assessed at baseline and 2 weeks and 3 months after the injections. The conclusions, disproportionate to the design, were that Intradetrusor injection of 200 U of BTX-A was effective in the treatment of detrusor overactivity that was refractory to anticholinergic agents and that the therapeutic effects lasted for 3 to 9 months (mean 5.3) [69].

An open, uncontrolled study in Scandinavia recruited fifteen patients suffering from NDO. The volume of urine leakage during episodes of incontinence was quantified, and filling cystometry was performed before and after BTX-A treatment. 300 IU of BTX-A was injected cystoscopically into the detrusor muscle, excluding the trigone region. The cystometry was interpreted as showing increase in bladder capacity and marked reductions in detrusor pressures after treatment, this being seen as proof of efficay. [70]. The

Laplacian relationship between pressure and volume (Pressure = 2 x Tension / Radius) means that if the bladder increases a contraction of similar force but at higher capacity will generate a lower pressure.

Researchers from Chicago University included thirty-five patients (29 women and 6 men) with idiopathic detrusor overactivity, who had failed anticholinergic therapy, in another open, uncontrolled study and injected 300 U of BTX-A injected transurethrally at 30 sites within the bladder. Patients were evaluated at 3 weeks and 6 months after treatment by completion of the short forms of the Incontinence Impact Questionnaire (IIQ-7) and the Urogenital Distress Inventory (UDI-6), as well as questions assessing global response to the treatment. A change in symptoms, associated with detrusor overactivity, that lasted for at least six months was reported and attributed to the drug.[71]

A lower dose of 150 U of Botulinum toxin A was used in an open, uncontrolled, study at the Duke University in North Carolina. Patients with evidence of urge urinary incontinence on 3-day bladder diary, a 24-hour pad weight of 100 gm or greater, absent or minimal stress leakage, absent detrusor dysfunction, and a history of failed anticholinergic and physical therapies were included. Evaluations were performed at 2 weeks, 6 weeks, 3 months and 6 months after injection and outcome measures included daily incontinence episodes, Urogenital Distress Inventory and the Incontinence Impact Questionnaire, 24-hour pad weights, daily pad usage and urinalysis at

all visits. They reported decreased urge urinary incontinence and improved quality of life for 3 months after injection [72]

A lower dose of 100U of BTX-A was used in an open uncontrolled study of 26 women with idiopathic detrusor overactivity (IDO) in Switzerland. BTX-A was injected into the detrusor muscle at 30 sites. Clinical and urodynamic evaluations and a quality of life assessment were performed at baseline and 4, 12, and 36 weeks after BTX-A treatment. Of 26 women, 14 were dry after 4 weeks, 13 of 20 women after 12 weeks, and 3 of 5 women after 36 weeks. Two women failed to respond.[73]

Schulte et al, in July 2005, investigated the effect of BTX- A bladder injections in the treatment of overactive bladder syndrome in the absence of detrusor overactivity. The patients were 7 women (average age 61.1 years, range 51 to 79) who presented with overactive bladder symptoms. Their disorder had been refractory to several classic treatment options. Urodynamic examination excluded detrusor overactivity. A total of 300 U BTX-A was injected, of which 50 to 75 U was injected as quadrant injections into the external sphincter muscle to avoid the postoperative need for catheterization in the case of high post void residual urine volume. For follow-up, complete urodynamic studies were performed, and a bladder diary and validated incontinence questionnaires were given to patients at all visits at 1, 3, and 6 months. The bladder diaries indicated a reduction in daytime frequency and nocturia and a reduction in pad use. The maximal voiding volume increased. The urodynamic

examinations showed an increase in volumes when the first and the strong desire to void were expressed. The maximal bladder capacity increased. In the questionnaires, 5 of the 7 patients reported better urine control after therapy, and 6 would have chosen this therapy again for their condition. This study was open and uncontrolled.[74]

Kuo et al. studied twenty patients with idiopathic detrusor overactivity refractory to anticholinergics. They were treated with injection of 200 U BTX-A into the suburothelial space and the clinical effects on the lower urinary tract symptoms and urodynamic variables were assessed. After these injections the 15 patients described hesitancy in initiation of voiding and difficult urination but also noted fewer OAB symptoms overactivity.[75]

A comparison between the response of patients with idiopathic detrusor overactivity and neurogenic detrusor overactivity to intradetrusor injection of BTX-A was made in a study at the Institute of Neurology, London. In this, open study, a total of 44 patients with spinal NDO and 31 with IDO who had urgency, and/or urgency incontinence, with detrusor overactivity on urodynamics, received 300 units (NDO) or 200 units (IDO) of BTX-A injected into the bladder with a minimally invasive outpatient technique. Urodynamic maximum cystometeric capacity and maximum detrusor pressure during filling, urinary frequency, incontinence episodes and urgency episodes from 4-day voiding diaries were compared between the 2 groups at baseline and for differences in change at 4 and 16 weeks after treatment. The authors

concluded that the patients with intractable IDO respond to intradetrusor BTX-A with similar changes in urodynamic variables and lower symptoms to those with spinal NDO. This was despite the lower dose of toxin used. [76]

Klaphajone et al reported a case series of ten patients with neurogenic detrusor overactivity combined with low-compliance bladder due to spinal cord lesions. 300U of BTX-A was injected into the detrusor muscle.. Urinary continence, functional bladder capacity, bladder compliance, detrusor contraction pressure, and volume at first reflex voiding were assessed. Measurements were taken before and 6, 16, and 36 weeks post treatment. Six weeks after treatment, complete continence was restored in 7 patients without oxyphencyclimine. Mean functional bladder capacity and compliance significantly increased, whereas maximal detrusor contraction pressure significantly decreased. Urodynamic variables remained significantly improved at 16 weeks, but values were returning toward baseline levels by 36 weeks.[77]

There is evidence that BTX-A may affect sensory fibres as well as motor. This was investigated in humans by studying the sensory receptors P2X3 and TRPV1 in detrusor biopsies from patients with neurogenic or IDO at the Hammersmith Hospital, London. Tissue was obtained at 4 and 16 weeks after injection with BTX-A from 38 patients (22 with neurogenic DO, 16 with idiopathic DO). Specimens were studied immunohistochemically for P2X3, TRPV1 and the pan-neuronal marker PGP9.5, in comparison with controls. P2X3-immunoreactive and TRPV1-immunoreactive (-IR) fibers were

decreased at 4 weeks after BTX-A, and more significantly at 16 weeks, when significant improvements were observed in clinical and urodynamic variables. P2X3-IR fiber decrease was significantly correlated with reduction of urgency episodes at 4 and 16 weeks, but not maximum cystometric capacity or detrusor pressures. TRPV1-IR fiber decrease showed a similar trend. PGP9.5-IR suburothelial fibers remained unchanged after treatment at both follow-ups.[78]. No control samples were included in the analysis.

The problem with all of these studies is that they did not use controls and the patients were not randomised. This situation usually arises because of the adoption of a treatment option in clinical practice that is then scrutinised post-hoc by retrospective analysis of the data. Some other factor may have led to the change in patient circumstance. A plausible argument offered in defence is that if open uncontrolled data be published from many different centres with complimentary results then the probability of accuracy increases. This is true but involves a small increase from a low starting probability.

The use of urodynamic as an outcome measure presents difficulties. In all the RCT published literature of the treatment of the overactive bladder where urodynamics has been used as an outcome measure the two variables which have been consistently reported to change for the better are the bladder capacity and the detrusor pressure. The change in detrusor pressure is a function of Laplacian physiology and therefore an epiphenomenon of the test. Bladder capacity can be assessed with a measuring jug and a frequency

volume chart. Where urodynamic findings have been compared with the patients experience of the disease correlations have failed to materialise.

The first randomised placebo-controlled trial measuring the safety and efficacy of BTX-A in neurogenic detrusor overactivity was published by Schurch et al in July 2005 in the report of a 6 month study. A total of 59 patients with urinary incontinence caused by neurogenic detrusor overactivity (due to spinal cord injury in 53 and multiple sclerosis in 6) requiring clean intermittent self-catheterization were randomised to receive a single dose into the detrusor of BTX-A (200 U or 300 U) or placebo. Changes in daily frequency of urinary incontinence episodes were monitored via a patient bladder diary during 24 weeks. Urodynamic measures of maximum cystometric capacity, reflex detrusor volume, and maximum detrusor pressure were used to assess treatment effect. Quality of life was assessed with the Incontinence Quality of Life questionnaire. There were significant post treatment differences in decreases in incontinence episodes from baseline in the 2 BTX-A groups compared to the placebo group. Positive treatment effects were also reflected by significant differences in change in bladder capacity and in patient quality of life. The differences in change were observed from the first evaluation at week 2 to the end of the 24-week study.[79]

1.8.2.2 Botulinum toxin and detrusor sphincter dyssynergia (DSD)

Sphincter injection of BTX A was first described in 5 patients with detrusor sphincter dyssynergia (DSD) by Dykstra et al in 1990. . The sphincter was injected with either a low dose of BTX A toxin or normal saline once per week for three weeks. Electromyography of the external urethral sphincter indicated denervation in the three patients who received toxin injections. The urethral pressure profile decreased an average of 25cm of water; post voiding residual volume of urine decreased an average of 125cc, and bladder pressure during voiding decreased to an average of 30cm of water. In the two patients who received normal saline injections, parameters were unchanged from baseline values until subsequent injection with BTX A toxin once per week for three weeks when their responses were similar to those of the other three patients. The duration of the toxin's effect averaged two months.

[80].

Schurch et al (1996) studied 24 spinal cord injury male patients with detrusor-sphincter dyssynergia. Transurethral and transperineal BTX A injections were performed and compared for efficacy. In 21 of 24 patients detrusor-sphincter dyssynergia was significantly improved with a concomitant decrease in post-void residual volumes in most cases. BTX A effects lasted 3 to 9 months.[81]

de-Seze et al conducted a double-blind lidocaine-controlled study in 13 patients with detrusor sphincter dyssynergia spinal cord disease.

Thirteen patients (1 female and 12 male) suffering from chronic urinary retention due to DSD were randomised to receive one transperineal injection of 100 IU BTX A or 4 ml of 0.5% lidocaine. The main criteria of efficacy were post-voiding residual urine volume, assessed three times daily on day one, day 7 and day 30 after each injection. Other criteria were micturition diary, satisfaction score, maximal urethral pressure, maximum detrusor pressure and type of DSD, recorded on day 0 and day 30. In, there were significant between group differences in change in post-voiding residual urine volume, maximal urethral pressure, favouring the BTX A group. Symptom score was higher in BTX-A group than Lidocaine group. [82]

A case series was reported from Russia on 9 patients (6 males and 3 females) with detrusor sphincter dyssynergia. Transperineal injection of 100 units of BTX- A was used under electromyographic control into the external urethral sphincter. Abdominal pressure fell from 75 to 39 cm, on the average; maximal detrusor pressure fell from 59 to 29 cm, and mean maximal urinary flow rate rose from 4.3 to 9.6 ml/s..[83]

An account of 6-years experience using BTX A in the bladder and urethra in 110 patients for a variety of lower urinary tract disorders was published in 2005. Of these 68 patients with detrusor sphincter dyssynergia were injected 100 to 200 U of BTX-A in 4 mL divided in equal doses into the four quadrants of the external sphincter, transurethrally. Two women with multiple sclerosis and mild baseline stress urinary incontinence reported increased leakage with

stress after BTX-A external sphincter injection, and one woman with multiple sclerosis noted new onset stress urinary incontinence after external sphincter injection. However, they all reported significant improvement in post void residual urine volume, uroflow, urge incontinence, and frequency.[84]

1.8.2.3 Paediatric uses of Botulinum toxin

The established treatment of children with neurogenic bladder consists of the use of anticholinergic drugs, such as oxybutynin and tolterodine, and clean intermittent catheterization four or five times a day.

Schulte et al in 2002 studied BTX A in children with detrusor hyperreflexia due to myelomeningocele. The case series were 17 children (average age 10.8 years) who had detrusor hyperreflexia and were using clean intermittent catheterization four or five times a day. Urodynamic studies were followed by injection of 85 to 300 U of BTX A into 30 to 40 sites in the detrusor muscle. Urodynamic follow-up was done 2 to 4 weeks after injection. A significant increase was seen in mean reflex volume, maximal bladder capacity and detrusor compliance. This was accompanied by a decrease in the detrusor pressures.[85, 86]

Further results were published by the same group in 2003. 20 Children with hyper-reflexive detrusor muscle and high bladder pressure, over 40 cm H_2O despite anticholinergic therapy were recruited for this study. After baseline

urodynamic measurements, they were injected BTX A into the detrusor muscle at 30-50 sites at 12 U/kg of body weight up to a maximum of 300 U. All the urodynamic parameters showed a significant improvement.[87]

Recently, Schulte et al assessed the long-term success of treatment with repeated BTX A injections into the detrusor muscle for neurogenic detrusor overactivity in children. The charts of 10 children (average age at first injection 11.2 years) with neurogenic detrusor overactivity were reviewed who had received at least three BTX A detrusor injections; four had received five or more injections. The total dose of BTX A was 85 to 300 U. d the urodynamic outcomes 6 months after each injection were measured and the results after the first injection compared with the results after the third and fifth injections. It was observed that there was a sustained improvement in all the urodynamic measures after repeated BTX A detrusor injections in children with neurogenic bladder. [88]

1.8.2.4 Botulinum toxin and benign prostatic obstruction

To expand the clinical use of BTX A in lower urinary tract dysfunction, an attempt has been made to study its clinical applications in benign prostatic hyperplasia (BPH).

In a prospective study, Kuo assessed the effectiveness of prostate injection of BTX A in patients who were poor surgical candidates. Ten patients with BPH and urinary retention or a large post void residual urine volume received 200 U botulinum A toxin injection into the transition zone of the prostate. The clinical results and urodynamic varaibles at baseline and after treatment were compared. All patients had an improvement in spontaneous voiding after treatment. Of them, 8 had an excellent result and 2 had an improved result. Both voiding pressure and post void residual volume were significantly decreased after treatment. The total prostate volume was significantly reduced, and the maximal flow rate was significantly increased after treatment. The maximal effects of BTX A appeared at about 1 week and were maintained at 3 and 6 months after treatment. At 6 to 12 months (mean 9) of follow-up, no patient had had recurrence of urinary retention and the voiding condition in all patients remained at the post-treatment status.[89]

Subsequently, sixteen men with symptomatic BPH in small prostates (prostate volume less than 30 cm3), peak flow rate less than 12 mL/s and with refractory disease after at least 1 month of alpha-blocker treatment received

BTX A 100 U injection into the prostate transperineally under transrectal ultrasound guidance. The clinical effects were evaluated at baseline and after treatment. All patients reported subjective improvement starting at approximately 1 week and achieved a maximal effect after 1 month that was maintained at 3 and 6 months. At 6 to 12 months (mean 10) of follow-up, no patient had symptom recurrence. The mean prostate volume, symptom score, and quality-of-life index were significantly reduced.[90]

1.8.2.5 Botulinum toxin and Interstitial cystitis

There is some evidence that BTX A might have analgesic properties. Chuang et al in 2004 investigated the effect of intravesical BTX A administration on afferent nerves of the bladder containing calcitonin gene-related peptide (CGRP) immunoreactivity. The study bladders were hyperactivity from an acetic acid induced bladder pain model in rats. Experimental and control animals were catheterized and intravesically exposed to protamine sulfate (1 ml, 10 mg/ml), followed by BTX A (1 ml, 25 U/ml) or saline, respectively. Three or 7 days after intravesical therapy continuous cystometrograms were performed using urethane anaesthesia by filling the bladder with saline, followed by 0.3% acetic acid. Bladder immunohistochemistry was used to detect CGRP. The intercontraction interval (ICI) was decreased after acetic acid instillation in the control group at days 3 and 7, respectively. However, rats that received BTX A showed a significantly decreased response to acetic acid instillation at day 7. This effect was not observed at day 3. Increased

CGRP immunoreactivity was detected in the BTX A treated group at day 7, which was not detected at day 3. Intravesical BTX A administration blocked acetic acid induced bladder pain responses and inhibited CGRP release from afferent nerve terminals. The authors made the claim that their data supported the clinical application of BTX-A for the treatment of interstitial cystitis.[91]

Smith et al presented clinical evidence from the use of BTX-A suggesting an antinociceptive role in patients with interstitial cystitis (IC). Thirteen female patients (6 in the United States and 7 in Poland) with IC according to the criteria of the National Institute of Diabetes, Digestive and Kidney Disease were included. 100 to 200 U BTX A was injected through a cystoscope into 20 to 30 sites submucosally in the trigone and floor of the bladder. Patients were evaluated with the O'Leary-Sant validated IC questionnaire or with voiding charts and a visual analog pain scale 1 month postoperatively and at subsequent 3-month intervals. Overall, 9 (69%) of 13 patients noted subjective improvement after BTX A treatment. The Interstitial Cystitis Symptom Index and Interstitial Cystitis Problem Index mean scores improved. Daytime frequency, nocturia, and pain by visual analog scale decreased and the first desire to void and maximal cystometric capacity increased. The authors proposed that BTX A may have had an antinociceptive effect on bladder afferent pathways in patients with IC, producing both symptomatic and functional improvements.[92]

By contradistinction, a recent report describes eight women and two men with chronic interstitial cystitis who had failed conventional treatments. They were enrolled in a study by Kuo in Taiwan. In 5 patients, 100 units of BTX A was injected suburothelially into 20 sites, and an additional 100 units were injected into the trigone in the other 5 patients. Therapeutic outcome including functional bladder capacity, number of daily urinations, bladder pain, and urodynamic changes were compared between baseline and 3 months after treatment. The clinical result of suburothelial BTX A injection was disappointing. None of the patients was symptom free and only a limited improvement in bladder capacity and pain score was achieved in 2 patients.[93]

Several publications have reviewed the applications of Botulinum toxin in the lower urinary tract. They conclude, on the available data, that intradetrusor injections of Botulinum toxin might become a standard therapeutic option in patients with neurogenic or non neurogenic detrusor overactivity, who do not respond to or do not tolerate anticholinergic medication. It might also be expected to improve bladder emptying in patients with dysfunctional voiding problems such as detrusor sphincter dyssynergia. However, the lack of placebo control and proper comparative design makes these conclusions optimistic.

To reach firmer conclusions, controlled studies with well defined patient populations and using validated and reproducible outcome measures are needed. In addition, information on repeated injections over a longer period of time is required.[94-106]

1.9 BOTULINUM TOXIN B

1.9.1 UNIQUE FEATURES

The Botulinum toxin B evokes particular interest because of the following:

- A Smaller molecular weight (5000-7000) as compared to Botulinum toxin A (9000)
- High stability at room temperature (25^{o})
- It does not need to be lyophilised (theoretically more potent)
- More rapid onset of action
- More even diffusion

} As compared to Botulinum toxin A

- It provides a useful alternative to Botulinum toxin type A in primary and secondary non responders i.e. the patients who develop immunity against the type A toxin because of repeated injections.
- Owing to its smaller size and different diffusion characteristics there is a theoretical possibility of delivering this drug by some other means rather than injections.
- Last but not the least, there is a chance of it being more cost effective because the properties may allow the use of less at a reduced frequency of injections[18, 107].

1.9.2 Botulinum toxin B and LUTS

Botulinum toxin type B (BTX B) has been little studied in lower urinary tract symptoms. The unique immunogenicity of the various serotypes of the toxin, along with the fact that both toxins interact with different target proteins means that BTX B has therapeutic potential in patients unresponsive to BTX A particularly those who have become resistant after repeated injections, consequent upon the antibody response [108, 109].

The first urological use of BTX B was reported as a case study published by Dykstra et al in which a patient with detrusor hyperreflexia was successfully treated with 2 separate injections of 5000U and 7500U of BTX B into the detrusor. The result of each treatment lasted for four months [110].

An open label dose escalation study of 15 female patients with overactive bladder demonstrated average action duration of 43 days with 5000U. The BTX B doses used in this study were 2500, 3750, 5000, 10000 and 15000 units. All but one patient responded with decreased frequency, urgency and no incontinence. The degree of response was similar across all doses; however, the duration of response was dose dependent [111].

Recently, a case report by Reitz and Schurch demonstrated successful management of two cases of neurogenic detrusor activity with BTX B injections who were resistant to BTX A [109].

In another study, done on the effects of Botulinum toxins in the treatment of palmar and axillary hyperhydrosis, it was shown that type B clearly produces a more rapid onset of effect than type A, and appears to diffuse more evenly in some injected muscles. This may permit a reduction in the number of injection sites required and may provide a smoother and more even clinical effect in some areas [112].

CHAPTER - 2

Chapter 2 DETRUSOR OVERACTIVITY AND VARIOUS TREATMENT MODALITIES

2.1 ICS NOMENCLATURE

Since all the patients recruited in our study had detrusor overactivity, it is necessary to discuss the nomenclature related to this condition.

In everyday life the individual attempts to inhibit detrusor over activity until he or she is in a position to void. Therefore, during cystometery, when the aims of the filling study have been achieved, and when the patient has a desire to void, normally the 'permission to void' is given. That moment is indicated on the Urodynamic trace and all detrusor activity before the permission to void is defined as 'involuntary detrusor activity'.

Normal detrusor function allows the bladder filling with little or no change in pressure. No involuntary phasic contractions occur despite provocation.

Detrusor overactivity is a urodynamic observation characterised by involuntary detrusor contractions during the filling phase which may be spontaneous or provoked. This may also be qualified, when possible, according to cause, as:

Neurogenic detrusor overactivity, earlier known as detrusor hyperreflexia, when there is a known neurological condition.

Idiopathic detrusor overactivity, earlier known as detrusor instability, when there is no defined cause.

Other relevant terms that are often used in this context are:

Phasic detrusor overactivity is defined by a characteristic waveform and may or may not lead to urinary incontinence.

Terminal detrusor overactivity is defined as a single, involuntary detrusor contraction, occurring at cystometeric capacity, which cannot be suppressed and results in incontinence usually resulting in bladder emptying.

Detrusor overactivity incontinence is incontinence due to an involuntary detrusor contraction [113].

2.2 DETRUSOR OVERACTIVITY-MANAGEMENT

There are several different approaches that may be used in managing and treating urge incontinence. If evidence of infection is found in urine culture, antibiotics will be prescribed. The choice of a specific treatment will depend on the severity of the symptoms and the extent that the symptoms interfere with your life style. There are three main approaches to treatment: medication, retraining, and surgery.

2.2.1 MEDICATION

Medications used to treat urge incontinence are aimed at relaxing the involuntary contraction of the bladder and improving bladder function. There are several types of medications that may be used alone or in combination:

- ✓ anticholinergic agents (oxybutynin, tolterodine)
- ✓ tricyclic antidepressants (imipramine)

Oxybutynin and tolterodine are antispasmodic medications that relax the smooth muscle of the bladder. These are the most commonly used medications for urge incontinence and are available in a once-a-day formulation that makes dosing easy and effective[114-121]. Side effects of oxybutynin and tolterodine are minimal, with the most common being dry mouth and constipation. However, these medications cannot be used by patients with narrow angle glaucoma.

Anticholinergic medications block inappropriate contractions of the bladder. They were widely used in the past to treat urge incontinence because they are relatively inexpensive yet effective. Oxybutynin and tolterodine have virtually replaced the use of these medications because they have fewer side effects [122-125].

Tricyclic antidepressants have also been used to treat urge incontinence because of their ability to inhibit or "paralyse" the bladder smooth muscle. Possible side effects include fatigue, dry mouth, dizziness, blurred vision, nausea and insomnia [119, 121, 126].

2.2.2 SURGERY

When conservative and pharmacologic treatments for detrusor overactivity incontinence fail, there are a number of surgical procedures that can be performed for the patient who is significantly bothered by his or her symptoms. Surgical treatments include sacral nerve neuromodulation, bladder denervation, autoaugmentation and bladder augmentation (Ileocystoplasty)[127-133].

Sacral Neuromodulation

Sacral nerve stimulation is performed in two stages: stage I, a clinical trial of a temporary or permanent lead for external stimulation; and stage II, implantation of a subcutaneous implantable pulse generator (IPG). Each stage can be performed with monitored anaesthesia care supplemented by local anaesthesia. During the initial introduction of sacral neuromodulation therapy, patients underwent a percutaneous nerve evaluation by the placement of a unilateral percutaneous lead in the S3 foramen with use of local injectable anaesthesia. The lead was connected to an external pulse generator and worn by the patient for several days. A large number of false-negative results with therapy are attributed to improper lead placement and migration. Whereas some physicians still prefer to perform the first stage by a percutaneous nerve evaluation approach, most have adopted a permanent

lead placement for the first stage in an attempt to avoid the issues related to high false-negative results with the first stage and high false-positive results with the second stage. Changes in lower urinary tract symptoms and post void residuals are recorded in a detailed bladder diary. If improvement is minimal or absent, revision or bilateral percutaneous lead placement may be attempted. If more than 50% improvement in symptoms of urgency-frequency or urge incontinence is attained, a permanent IPG is implanted. The length of the trial with the external pulse generator may vary slightly from patient to patient, by the indication, and by the surgeon's practice preference.

Schmidt and co-workers (1999) reported on SNS therapy in 76 patients with refractory urge incontinence from 16 centres worldwide randomized to active or delayed therapy (control group) during the study period of 6 months. Of the 34 patients receiving active SNS therapy compared with the delayed group, 16 (47%) were completely dry, and an additional 10 (29%) demonstrated more than 50% reduction in incontinence episodes. Complications were IPG site pain in 16%, implant infections in 19%, and lead migration in 7%[134].

In a similar study design, Hassouna and colleagues (2000) reported the outcomes of SNS on refractory urgency-frequency conditions in 51 patients randomized from 12 centres during an initial 6-month period that was extended to 2 years. Outcomes at 6 months in the active SNS group showed improvement in the number of daily voids (16.9 ± 9.7 to 9.3 ± 5.1), volume voided (118 ± 74 mL to 226 ± 124 mL), degree of urgency (rank score of 2.2 ± 0.6 to 1.6 ± 0.9), and SF-36 quality of life measures. At 6 months after

implantation, stimulators in the active group were turned off and urinary symptoms returned to baseline values. After reactivation of SNS, sustained efficacy was documented at 12 and 24 months[135].

Denervation Procedures

Denervation techniques include bladder transection and reattachment, either by open surgery or endoscopically; complete S2 to S4 rhizotomy; partial rhizotomy; and subtrigonal phenol or alcohol injections. There is little documentation of long-term efficacy of these denervation procedures, and many of the techniques are associated with considerable morbidity, and, for practical purposes, they have been abandoned[136-139] Furthermore, despite an initial short-term response, the long-term results are often dominated by the development of low bladder compliance or recurrent detrusor overactivity. Sacral rhizotomy is reserved for the paraplegic or quadriplegic population. Posterior rhizotomy may be combined with the placement of a neurostimulator on the anterior nerve roots to inhibit involuntary contractions and stimulate spontaneous voiding. In 1959, Ingelman-Sundberg described a transvaginal technique intended to accomplish partial denervation of the subtrigonal nerve supply to the bladder by selectively dividing the preganglionic nerves near the inferior surface of the bladder through a transvaginal incision. Several authors have reported success [140 -141], but outcome measures have included mostly patients' assessments done in a retrospective fashion. Thus, the procedure has never gained acceptance.

Autoaugmentation of the bladder

Autoaugmentation of the bladder was initially described by Cartwright and Snow (1989) as an alternative to enterocystoplasty in children with neuropathic bladder[142].

Autoaugmentation may be performed by incision (detrusor myotomy) or excision (detrusor myectomy) of a portion (usually the anterior, lateral, and superior surface) of the detrusor muscle. Either technique creates a mucosal bulge or pseudodiverticulum, which should increase capacity and decrease storage pressure.

There have been several reports of successful short-term outcomes after detrusor myectomy in children[142] and in adults with neuropathic bladder [143]. However, longer term follow-up in children [144] and adults with non-neurogenic urge incontinence [145]showed disappointing results.

Augmentation Cystoplasty

Augmentation cystoplasty increases bladder capacity and decreases detrusor overactivity by enlarging the bladder with the addition of a bowel segment and possibly by disrupting the detrusor. In a large majority of patients with refractory detrusor overactivity, augmentation enterocystoplasty is effective, provided that these basic principles are followed: (1) the intestinal segment is detubularized by incising the antimesenteric border, (2) the segment is reconfigured into the approximate shape of a half-sphere, (3) a wide anastomosis between the reconfigured bowel and the bladder is performed, and (4) a large bladder capacity is achieved. Classically, augmentation enterocystoplasty has been performed in patients with neurogenic voiding dysfunction, but it has been shown to be effective for patients with neurogenic and non-neurogenic detrusor overactivity [146].

Many patients, however, require intermittent self-catheterization after augmentation and need to be prepared to do this prior to consenting to the operation[147-148].

Complications of augmentation cystoplasty include metabolic disturbances (hyperchloremic acidosis), urinary tract infections, stone formation, perforation, and malignancy.

2.2.3 DIET

Some experts recommend a regimen of controlled fluid intake as a supplement to other therapies in the management of. The goal of this program is to distribute the intake of fluids throughout the course of the day, so the bladder does not need to handle a large volume of urine at one time.

Fluid restriction has been advocated in the treatment of both SUI and OAB. The rationale is that abdominal leak pressures appear to be volume dependent; therefore, physical stress occurring at lower bladder volumes will be both less likely to cause incontinence and will be associated with lower volume loss when leakage does occur. Similarly, OAB is believed to be a volume-driven phenomenon, and slower filling promotes bladder compliance and lower pressures. This concept appears to be well accepted, as manifest by a recent U.S. study in which 38% of incontinent women had tried limiting fluids compared with 21% who tried Kegel exercises and 6% using prescription medications [149]. On the other hand, extreme fluid restriction produces concentrated urine, which has been postulated to be a bladder irritant, leading to detrusor overactivity as well as constipation, which can negatively affect bladder function. Indeed, there is conflict regarding fluid management, with some investigators showing improvement with fluid reduction, whereas others finding that increasing fluid intake improved incontinence. It would appear that it is best to recommend a normal fluid consumption, reserving fluid restriction for those patients with abnormally high fluid intakes [150].

Caffeine is well known as a nervous system stimulant and has demonstrable effects on detrusor muscle in vitro and in vitro, promoting unstable contractions. In one study of community-dwelling elderly women subjects who decreased caffeine and increased fluid intake, increased voiding volumes and fewer accidents were experienced [151]. High caffeine intake (>400 mg/day average) also correlated with urodynamic detrusor overactivity compared with stress-incontinent women (<200 mg/day average) [152]. It has thus been postulated to be a cause of OAB symptoms, and caffeine reduction has been advised for OAB patients. Epidemiologic data are less clear. Reports usually break down consumption by type of beverage rather than actual caffeine consumption as is typically found in smaller studies of dietary intervention. Such small studies have typically shown a correlation between caffeine reduction and reduction of incontinence [151,153]. Although larger studies would be helpful, it seems appropriate to recommend restriction, particularly in those patients with very high intake of caffeine.

Tea consumption correlated with urge incontinence in the EPINCONT study, but alcohol and coffee consumption did not [154]

Additionally, it may be helpful to eliminate your intake of foods that may irritate the bladder, such as caffeine, spicy foods, carbonated drinks, and highly acidic foods such as citrus fruits and juices [151,153,155,157,158,159,].

2.2.4 BLADDER RETRAINING

Management of bladder over activity usually begins with a program of bladder retraining. Occasionally, electrical stimulation and biofeedback therapy may be used in conjunction with bladder retraining.

A program of bladder retraining involves becoming aware of patterns of incontinence episodes and relearning skills necessary for storage and proper emptying of the bladder. Bladder retraining alone is successful in 75% of people treated for urge incontinence.

Bladder retraining consists of developing a schedule of times when you should try to urinate, while trying to consciously delay urination between these times. One method is to force yourself to wait 1 to 1 1/2 hours between urinations, despite any leakage or urge to urinate in between these times. As you become skilled at waiting, gradually increase the time intervals by 1/2 hour until you are urinating every 3 to 4 hours [159-164].

Some therapists place a sensor in the vagina (for women) or the anus (for men) to assess contraction of the pelvic floor muscles. A monitor will display a graph showing which muscles are contracting and which are at rest. The therapist can help you identify the correct muscles for performing Kegel exercises[165-167].

About 75% of people who use biofeedback to enhance performance of Kegel exercises report symptom improvement, with 15% considered cured[168,169].

Electrical stimulation involves using low-voltage electric current to stimulate the correct group of muscles. The current may be delivered using an anal or vaginal probe. The electrical stimulation therapy may be performed in the clinic or at home. Treatment sessions usually last 20 minutes and may be performed every 1 to 4 days.

Some clinical studies have shown promising results in treating urge incontinence with electrical stimulation.

2.2.5 ACTIVITY

There are now epidemiologic data supporting a causal association between obesity and incontinence. In a 1-year longitudinal study of 6424 women older than 40 years of age there was a strong correlation between body mass index (BMI) and the risk of both OAB and SUI [170] .

Other studies consistently confirm the relationship between obesity and Urge Incontinence[171,172].

There are positive case series of morbidly obese women but only one published, peer-reviewed randomized study investigating the effect of weight loss on UI. Subak and colleagues (2005) randomized 48 women with UI and BMI between ages 25 and 45 to receive a liquid diet weight reduction program either immediately or after a 3-month delay. The median weight (interquartile range)) weight was 97 kg (87 to 106 kg), and the incontinence frequency was 21 episodes per week (11 to 33). At the end of 3 months women in the immediate treatment group lost an average of 16 kg compared with 0 kg in the delayed group. Incontinence episodes decreased 60% in the treatment group compared with 15% in the controls. Improvement was seen in both SUI and UUI. A similar response was then seen when the delayed group was treated. This study suggests that all types of UI may respond to moderate weight loss[173].

CHAPTER - 3

Chapter 3- METHOD

3.1 DISCUSSION OF METHOD

Patients with detrusor over activity represent quite a large population of urology clinic attenders. A number of medications and treatment modalities have been found to be effective for many of these. Nevertheless others fail to achieve a satisfactory response and alternatives therefore continue to be sought.

3.2 PROPOSED TREATMENT OF STUDY

Botulinum toxin is a highly potent neurotoxin, the therapeutic effects of which are principally derived from an alteration in the release of acetylcholine at presynaptic junction neurons. Botulinum toxin is approved for treatment of muscle over activity associated with several disorders, such as cervical dystonias. Clinical trials of its use in cases of detrusor over activity have shown encouraging results, both in Europe and America.

3.3 METHOD CONSIDERATIONS

Historically, urodynamics has been used as the primary outcome measure in the assessment of new treatments for the overactive bladder. However, scrutiny of the data has demonstrated that this is not the reliable option that was assumed. It has been found that urodynamic variables do not relate to the severity of a patient's symptoms, the prognosis nor the outcome of treatment[174]. A recent randomised, double-blind, placebo controlled study of tolterodine for detrusor instability has demonstrated urodynamics to play no appreciable role in the assessment process[175,176].

Nevertheless, it would not be advisable to diagnose patients for this study on symptoms alone since this could allow for some doubt as to the true nature of the aetiology. It is therefore more appropriate to describe the clinical pathology in terms of urodynamics by making the demonstration of detrusor instability a recruitment criterion.

It is now well accepted that Quality of Life (QOL) is an important outcome measure. The International Continence Society (ICS) has recommended that QOL measurements be included in all studies of urinary incontinence as a complement to clinical measures. Patients with detrusor over activity have QOL scores significantly worse than the general population[177-182]

Several QOL measures have been developed, validated and used extensively. There are a few specifically for urinary incontinence, including the Incontinence Impact Questionnaire, the York Incontinence Perception Scale (YIPS), the Incontinence Quality of Life (IQOL), the Incontinence Quality of Life index (IQLI) and the King's Health Questionnaire (KHQ) [183-187].

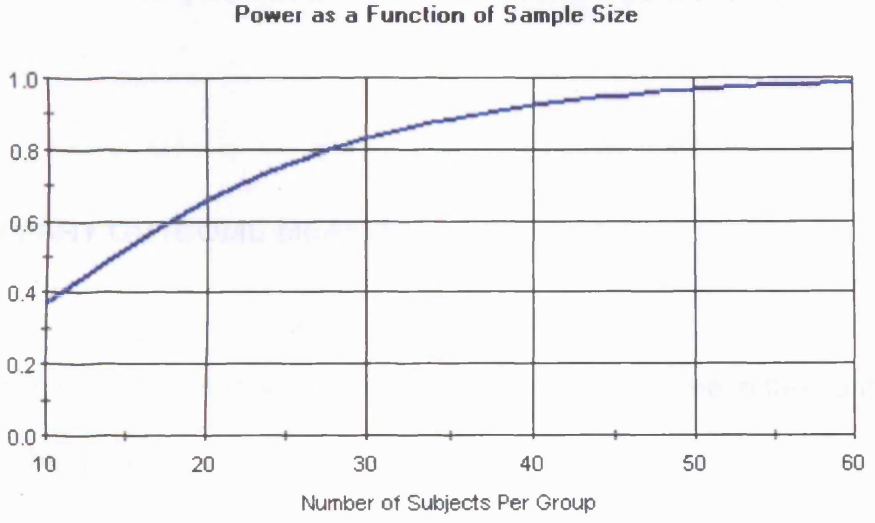
Of them all the KHQ is by far the better validated and has been shown to identify a change in response to treatment. On its history of use and accumulated experience it presents as the ideal choice from the field[188-191].

3.4 STUDY DESIGN

A matter of significant concern is the nature of the trial limbs. The major multi-centre clinical trials of oral preparations for treating incontinence have used parallel groups. These work well but require large recruitment frames and the participation of many centres. Cross-over deigns have been used very successfully in contexts similar to those considered in this study. Recent work on the use of intra-vesical atropine for the treatment of incontinence in MS, funded by the MS Society, has used cross-over methods with great success, achieving highly powered studies. There are two reasons for this. The type of patients requiring more advanced treatment methods exhibit detrusor overactivity that is highly reactive such that the discontinuation of oral antimuscarinic agents is associated with a very rapid decline in symptoms. This means that carry-over effects that normally prohibit cross-over do not feature. Secondly intravesical applications are associated with much greater increases in functional bladder capacity than oral preparations. Both atropine and oxybutynin have exhibited this property and the current data available on botulinum toxin indicates that the same properties feature. This results in a very clear discrimination between treatment effect and placebo, or non-treatment that is not only permissive of a cross-over design but also allows for highly powered studies from quite small sample numbers. For example the pilot study of intravesical atropine in MS demonstrated a difference in change in bladder capacity that was both clinically and statistically significant with a sample of 15 patients. Drawing on these experiences we proposed a

randomised, double-blind, placebo controlled, cross-over design identical to that used by Deaney et al in 1998[192].

As per the sample size computation 40 patients in each group would give 92% power and 30 in each group would give 83% power.



3.5 TRIAL OBJECTIVES AND ENDPOINTS

AIM

To test the hypotheses that the mean difference in change of average voided volume, following Botulinum B injection, compared with that following placebo is zero.

PRIMARY OUTCOME MEASURE

To compare the changes in the average voided volume in the control and the placebo group.

SECONDARY OUTCOME MEASURE

- Frequency
- Incontinence episodes
- Quality of life (Kings Health Questionnaire)
- To compare the differences in change in general quality of life

3.6 TRIAL DESIGN

A randomized, double-blind, placebo controlled, cross over trial comparing botulinum toxin B with placebo.

INCLUSION CRITERIA

- Male or female patients aged at least 18 years
- Patients able and willing to attend clinics
- Patients able and willing to complete diary card, including proper voided volume measurements for at least 48 consecutive hours.
- Patients able to visit toilets without any assistance.
- Patients capable of understanding the research nature of treatment and the risk of adverse events, and having signed the informed consent after full discussion.
- Patients with neurological or non neurological bladder instabilities proven by urodynamics.

EXCLUSION CRITERIA

- TCC of the bladder or any other bladder malignancy
- Previous bladder surgery (viz. augmentation cystoplasties)
- Active UTI

- Known allergies
- Children
- Known prostatic cancer
- Major urethral access problems

3.7 PRACTICAL METHOD

1. Patients were recruited after having a standard static saline fill cystometry with a filling rate of 50ml/minute
2. Patients were handed a voiding diary and a quality of life questionnaire at the time of recruitment. These forms are simple to complete. Instructions were clear.
3. Patients reported to the day case surgery unit.
4. A urine dipstick was performed to test for leucocyte esterase as evidence of infection.
5. A cystoscopy was performed and three biopsies were obtained from the dome of the bladder.
6. Each patient was injected with either 5000U of Botulinum toxin B (diluted in 20mls. Of saline) or 20mls of placebo, as allocated, intravesically. The drugs were prepared by a nurse and both the surgeon and the patient were blinded. The test drug was injected in equal aliquots over the dome of the bladder, excluding the trigone, at 10 different sites.
7. Patients went home the same day with a voiding diary and a questionnaire. They were prescribed 5 days of Ciprofloxacin 500mg X BD to cover for any infections.
8. Patients had a telephonic follow-up after one week to check for any side effects.

9. Patients maintained records of frequency, incontinence and voided volumes for three days continuously each week.
10. Patients reported to the day case surgery at the end of six weeks, with the completed voiding diary and a questionnaire, for cystoscopy and bladder biopsies. They received the crossover allocation of the study drug at this time, injected as previously into the bladder wall at ten sites.
11. Patients went home the same day with a new voiding diary and a questionnaire. They were prescribed five days of Ciproxin 500mg BD to cover for any infections.
12. Patients had a telephone follow-up at the end of one week to check for any side effects.
13. At the end of six weeks, patients were seen in the outpatients' clinic, with the completed voiding diary and a questionnaire to assess their symptoms and progress.

	Week 1								
	Day1 Date _____ Day _____			Day2 Date _____ Day _____			Day3 Date _____ Day _____		
	toilet (ml)	catheter (ml)	wet	toilet (ml)	catheter (ml)	wet	toilet (ml)	catheter (ml)	wet
6-7am									
7-8am									
8-9am									
9-10am									
10-11am									
11-12am									
12-1pm									
1-2pm									
2-3pm									
3-4pm									
4-5pm									
5-6pm									
6-7pm									
7-8pm									
8-9pm									
9-10pm									
10-11pm									
11-12pm									
12-1am									
1-2am									
2-3am									
3-4am									
4-5am									
5-6am									
Totals									

Figure 3.1
Voiding Diary

Bladder symptom questionnaire

Name.....
Date of birth.....
Sex F / M.....
Hospital number.....

	None	Some	Much	
	☺	😐	☹	
Do you experience urgency? That is having to hurry in order to pass urine? →→→→→→→→→	☺	😐	☹	1
Do you experience urge incontinence? That is hurrying to pass urine and not making it in time? →→→→→→→→→	☺	😐	☹	2
Does cold weather make your bladder symptoms worse? →→→→→→→→→	☺	😐	☹	3
Do you find that running water from a tap causes urinary urgency or incontinence? →→→→→→→→→	☺	😐	☹	4
Do you find that putting a key in the front door when returning home causes urinary urgency or incontinence? →→→→→→→→→	☺	😐	☹	5
Do you find that on getting up from bed in the morning you experience urgency or urge incontinence? →→→→→→→→→	☺	😐	☹	6
Is there any pain associated with the urgency? →→→→→→→→→	☺	😐	☹	7
Do you ever leak urine on: coughing, sneezing, running, laughing, lifting? →→→→→→→→→	☺	😐	☹	8

Date.....

Figure 3.2
Urgency symptom questionnaire

Kings Health Questionnaire

Present Health	Tick One
Very Good	
Good	
Fair	
Poor	
Very Poor	

Affect on Life	Tick one
Not at all	
A little	
Moderately	
A Lot	

	A Little	Moderately	A Lot
Frequency			
Nocturia			
Urgency			
Urge Incontinence			
Stress Incontinence			
Nocturnal Enuresis			
Intercourse Incontinence			
Frequent UTI			
Bladder Pain			
Difficult Micturition			

	Not at all	Slightly	Moderately	A Lot
Role Limitations (Impact on)				
Household tasks				
External tasks				
Physical and social Limitations (Impact on)				
Physical activities				
Travel				
Social Life				
Visiting Friends				
Personal Relationships (Impact On)				
Relationship with partner				
Sex Life				
Family life				
Emotions				
Depression				
Anxious or Nervous				
Impact on self esteem				
Sleep or energy				
impact on sleep				
Tiredness				

	Never	Sometimes	Often	All the times
Wear Pads				
Watchful fluid intake				
Change underclothes				
Worry in case you smell				
Embarrassment				

Figure 3.3
King's Health questionnaire

3.8 PRACTICAL PROCEDURES AFFECTING THE PATIENT

3.8.1 THE URODYNAMIC METHOD

Patients were asked to empty their bladders.

A Nelaton Jacques catheter (French gauge 10) and a nylon epidural catheter 916g) were introduced into the bladder via the urethra, which had been anaesthetised by 2% lignocaine gel.

The post micturition residual urine was drained off and its volume measured.

Another P.V.C catheter (French gauge 10) tipped with perforated latex sheath, to avoid faecal plugging, was introduced into the rectum.

The small bladder catheter and rectal catheter were filled with normal saline and then connected to force displacement transducers mounted at the level of the superior ramus of the pubic bone. This reference point was used to calibrate the transducers to atmospheric pressure.

The intrinsic detrusor pressure generated by the walls of the bladder, was calculated by subtracting the intra abdominal pressure (measured via the rectal catheter) from the bladder pressure (measured via the epidural catheter).

The bladder was filled with normal saline at 20°C at a rate of 1ml sec^{-1}. The analogue data obtained from the transducers were digitised and collected by micro-computer and stored on magnetic disk at a rate of 6 cycles sec^{-1}.

The bladder was filled until either a maximum of 500ml had been infused; or detrusor overactivity prohibited further filling; or the patient was found to be unable to tolerate further infusion.

On completion of the filling study the Nelaton Jacques catheter was withdrawn from the bladder and the patient was then asked to void to completion.

The digitised data was analysed so as to calculate the study variables.

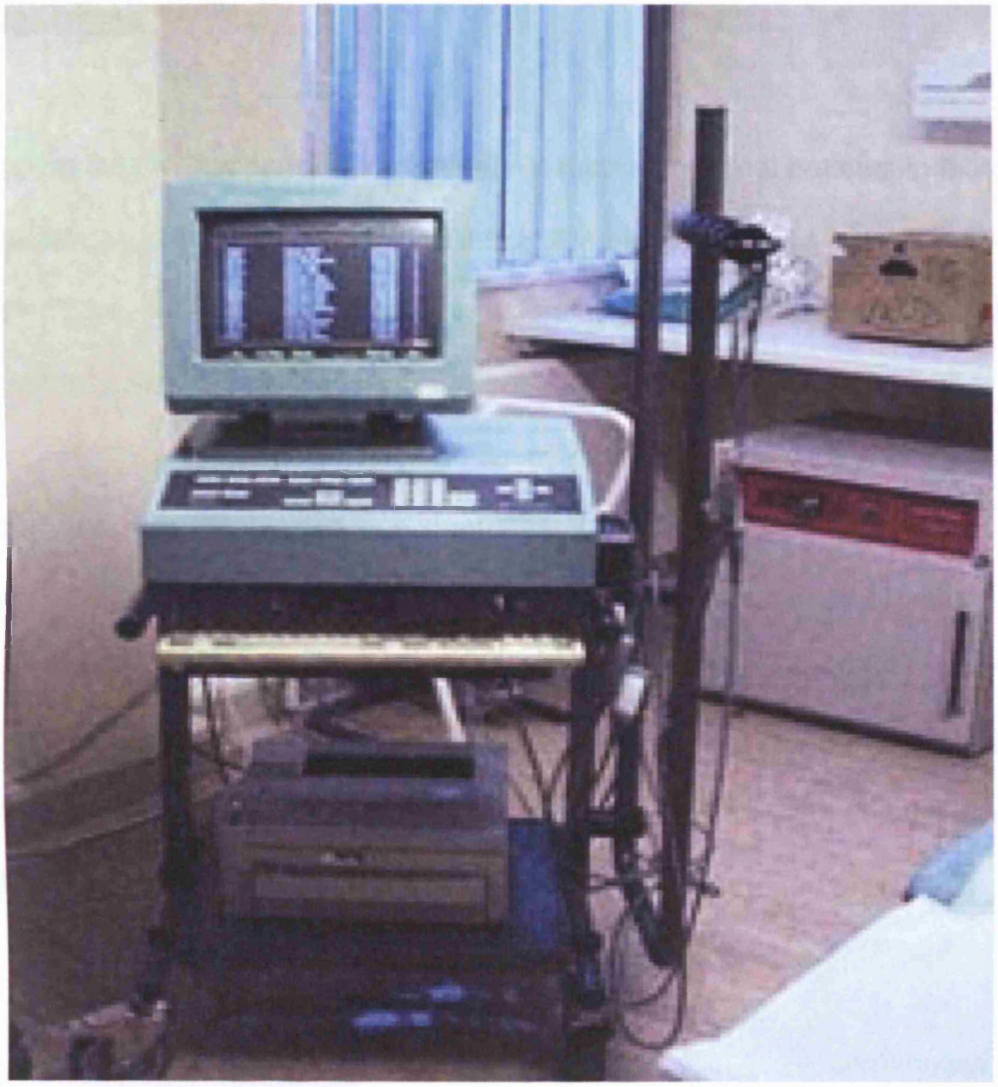


Figure 3.4
The Urodynamics equipment.

3.8.2 CYSTOSCOPY AND BLADDER BIOPSIES UNDER SEDO-ANALGESIA

This procedure was performed routinely in many urological patients in the day case surgery unit. It involved a look inside the bladder using a Millerscope (size17) under cover of mild sedation using intravenous midazolam. This has been termed as sedoanalgesia in the past[193-197].

Midazolam was used to produce sleepiness or drowsiness and to relieve anxiety before surgery or certain procedures. It was also used to produce sleep before and during surgery. Midazolam was used sometimes in patients in intensive care units in hospitals to cause sleep. This allowed the patients to withstand the stress of being in the intensive care unit. Midazolam was given only by or under the immediate supervision of a doctor trained to use this medicine.

The dose of midazolam was be different for different patients. The dose depending on:

- Age;
- Weight;
- General physical condition;
- The kind of procedure
- Other medicines the patient is taking or will receive before and during the procedure

Along with its needed effects, a medicine may cause some unwanted effects. While patients were receiving midazolam the doctor monitored closely for the side effects. The following is a complete list of side effects of midazolam:

Fluctuations in respiratory rate, including decreased respiratory rate and tidal volume, apnoea, variations in blood pressure and pulse rate are common. The following are general side effects regardless of the route of administration.

Cardiovascular: Hypotension, cardiac arrest.

Nervous system: Oversedation, headache, drowsiness, grogginess, confusion, retrograde amnesia, euphoria, nervousness, agitation, anxiety, argumentativeness, restlessness, emergence delirium, increased time for emergence, dreaming during emergence, nightmares, insomnia, tonic-clonic movements, ataxia, muscle tremor, involuntary or athetoid movements, dizziness, dysphoria, dysphonia, slurred speech, paresthesia.

Gastorinestinal: Hiccoughs, N&V, acid taste, retching, excessive salivation.

Ophthalmologic: Double vision, blurred vision, nystagmus, pinpoint pupils, visual disturbances, cyclic eyelid movements, difficulty in focusing.

Dermatologic: Hives, swelling or feeling of burning, warmth or cold feeling at injection site, hive-like wheal at injection site, pruritus, rash.

Miscellaneous: Blocked ears, loss of balance, chills, weakness, faint feeling, lethargy, yawning, toothache, and haematoma.

Some side effects may occur but it is very usual that they do not need medical attention. Most side effects will go away as the effects of midazolam wear off. The patients could therefore be discharged from the hospital the same day.

Biopsy specimens, which included detrusor muscle, were prepared for histopathological examination after fixation and H & E staining.

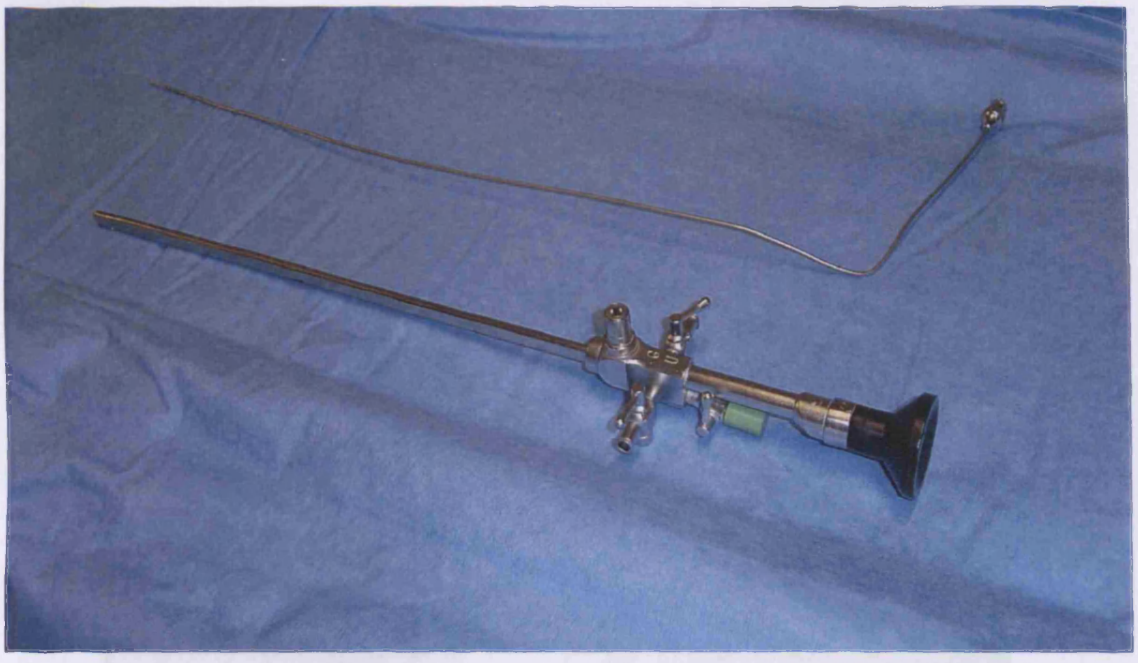


Figure 3.5
The Millerscope and the
semirigid Needle

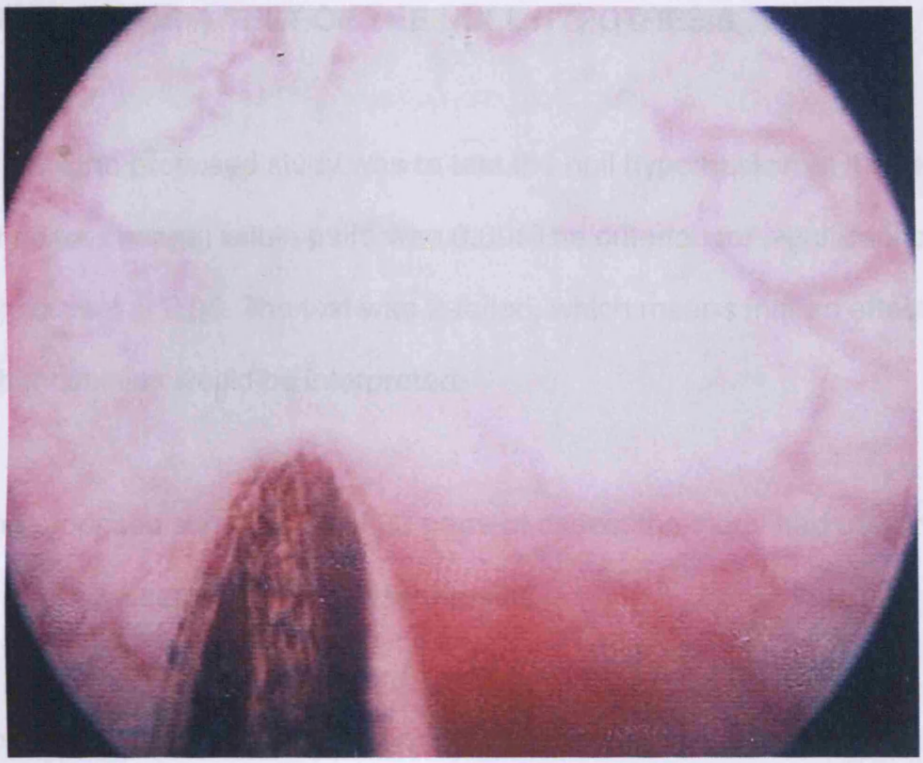


Figure 3.6
The Needle piercing the detrusor

3.9 STATISTICAL METHODS

3.9.1 POWER FOR A TEST OF THE NULL HYPOTHESIS

One goal of the proposed study was to test the null hypothesis that the mean difference (or change) within pairs was 0.00. The criterion for significance (alpha) was set at 0.05. The test was 2-tailed, which means that an effect in the either direction would be interpreted.

With the proposed sample size of 20 pairs of cases, the study had power of 86.3% to yield a statistically significant result.

The computation assumed that the population from which the sample was drawn exhibited a mean difference in average voided volume of 90 ml with a standard deviation of 125.0. The observed value was to be tested against a theoretical value (constant) of 0.00.

This effect was selected as the smallest effect that could be important to detect, in the sense that any smaller effect could not be of clinical or substantive significance. Rather than make an arbitrary judgement or guess at a suitable effect size we used the value of 90 ml (sd=125 ml) which had been found in a study of intravesical atropine. This study, which was conducted on patients with MS had found this difference in association with a marked

change for the better in the quality of life of the participating patients. We had evidence therefore that this change was of clinical significance.

We also assumed that this effect size was reasonable, in the sense that an effect of this magnitude could be anticipated in this field of research. On this point we assumed some similarity between responses to intravesical atropine and the intra-detrusor injection of botulinum toxin.

3.9.2 PRECISION FOR ESTIMATING THE EFFECT SIZE

A second goal of this study was to estimate the mean difference in the population. On average a study of this design would enable us to report the mean difference with a precision (95.0% confidence level) of +/- 57.47 points. For example, an observed mean difference of 90.0 would be reported with a 95.0% confidence interval of 32.53 to 147.47.

The precision estimated here is the median precision. Precision will vary as a function of the observed standard deviation (as well as sample size), and in any single study would be expected to be narrower or wider than this estimate.

CHAPTER - 4

Chapter 4 RESULTS

4.1 RECRUITMENT

Between April 2003 and August 2004, 30 patients were identified as eligible for the trial because of their symptoms.

Two were unwilling to participate. Eight patients did not show unstable contractions on urodynamics so they were not included in the study.
20 patients, 3 males and 17 females with a mean age of 50 (SD=16) entered the trial.

Of these, 3 female patients had neurogenic and 17 had non neurogenic detrusor overactivity.

No patient showed evidence of low compliance.

4.2 TRIAL PROFILE

10 patients received 5000IU of BTX B followed by the placebo six weeks later and the other 10 received placebo followed by 5000IU of BTX B (Figure 3.1).

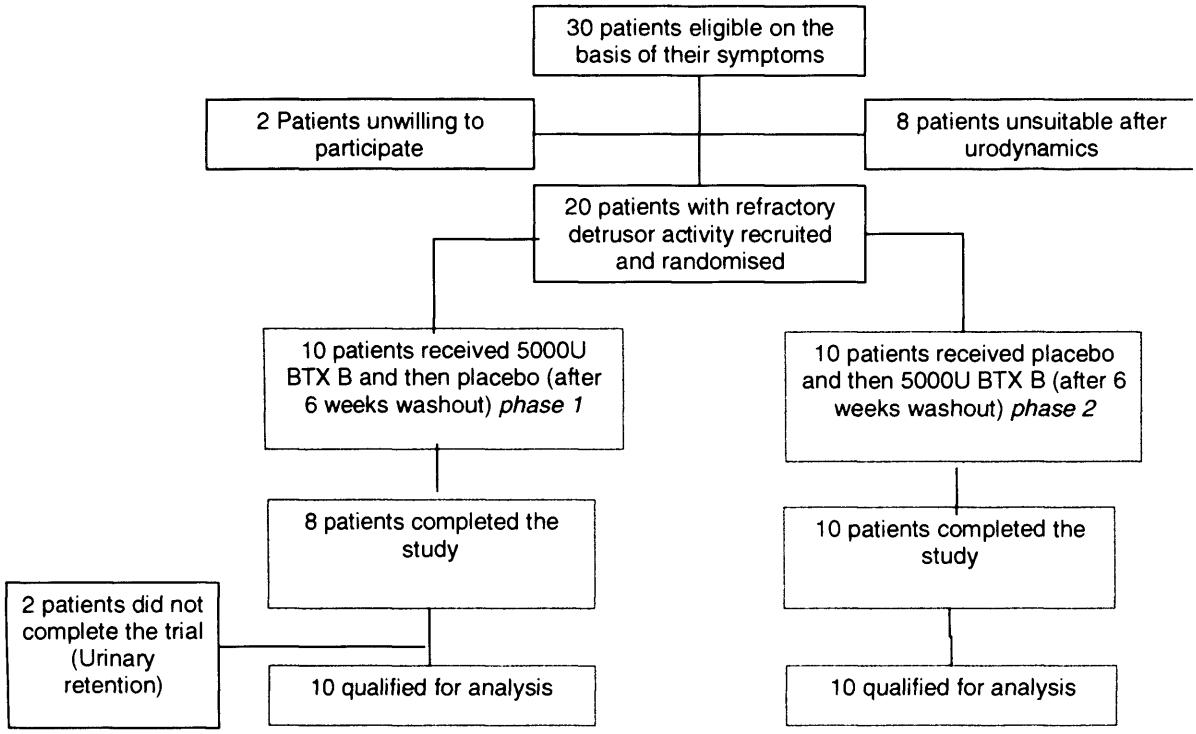


Figure 4.1
Trial Profile

4.3 RANDOMISATION AND BLINDING

All the patients were recruited by the lead researcher. A computerised random number generator was used by the clinical trials pharmacist, to draw up a randomisation schedule for treatment allocation. Identical packages were produced containing the trial medication (active/placebo) according to the randomisation schedule. The contents of these were known only to the clinical trials pharmacist. The syringes of the trial medication were prepared by the unblinded nurse, who was specifically assigned this task and had no other involvement with the trial or the patients.

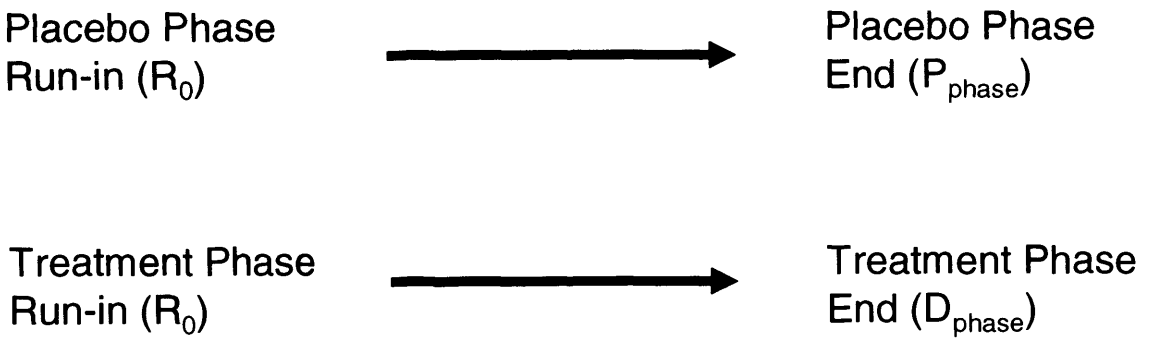
4.4 ANALYSIS

INTENTION TO TREAT

Two patients, who developed retention, provided absent data fields. Their most recent datasets were collected at baseline and these were copied into the gaps left in the spreadsheet.

SUBSET ANALYSIS (PER PROTOCOL)

A subset analysis on the basis of per protocol data was also effected where the data from the patients, who did not complete the trial, was excluded.
In addition to this another subset was identified. This was of the patients who did not have neurogenic detrusor overactivity ("Idiopathic detrusor overactivity"). These were 17 in number. An independent analysis was carried out for this subset.



Between Group Difference in Δ
Δ D - Δ P = **True Drug Effect**

Figure 4.2
Result Analysis

4.5 PRIMARY OUTCOME MEASURE- AVERAGE VOIDED VOLUME

The Wilcoxon Signed Ranks Test was used to test the paired difference in change between treatment phases. Little carry-over was noted in the second arm placebo and the placebo data from both arms were included in the analysis.

Paired differences, between treatment arms, in the changes from baseline were found for the primary outcome variable, average voided volume.
The median difference and the confidence intervals were sought:
Wilcoxon Signed Ranks Test Statistic Z = -2.5; p= 0.012; Median difference in change = + 65 ml 95% C.I. = +11, +121

The results are illustrated in Table 3.1. Figure 3.2 illustrates the median and 95% C.I. for the primary outcome measure during run-in, and the two treatment arms. The paired difference between active treatment and the other two phases is evident.

	Median	95% Confidence intervals	
		Lower	Upper
Baseline	126.7	92.1	171.1
Active	228.6	162.6	279.5
Placebo	151.4	98.9	187.2
Change from baseline on active treatment	83.0	6.1	132.4
Change from baseline on placebo	4.48	-4.05	43.43
Wilcoxon Signed Ranks Test Statistic Z = -2.5; p= 0.012; Median difference in change = + 65 ml 95% C.I. = +11, +121			

Table 4.1
Median of absolute values and their change for average voided volume

Figure 2

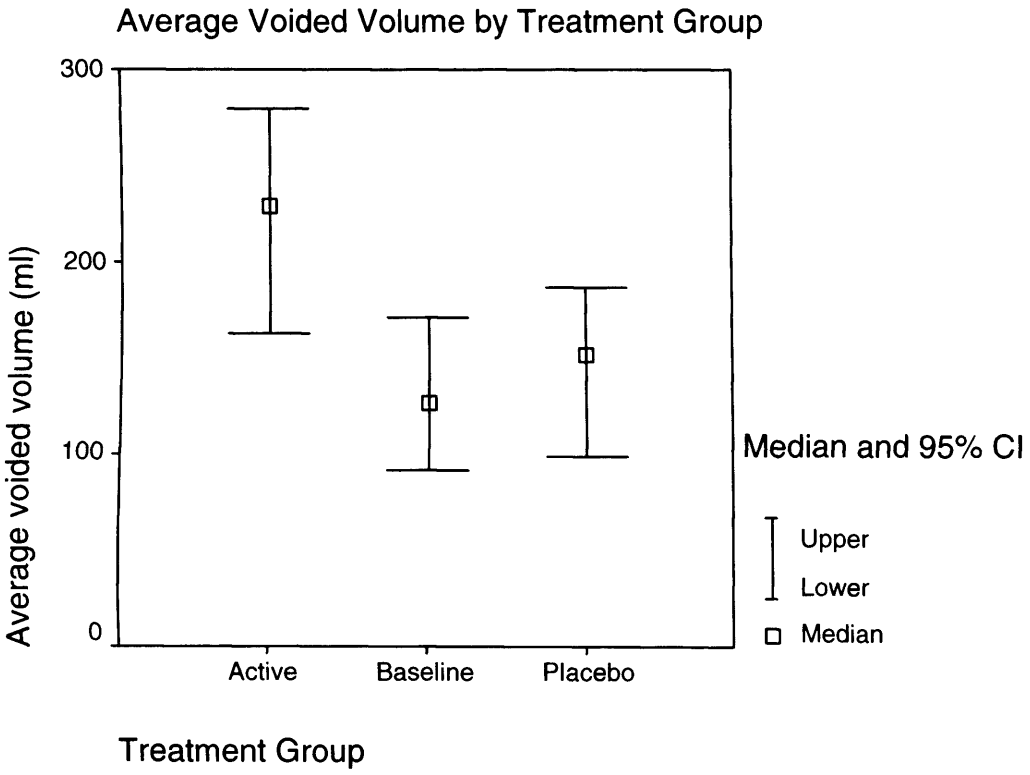


Figure 4.3

The median and 95% C.I. for the primary outcome measure during run-in, and the two treatment arms

4.6 SECONDARY OUTCOME MEASURES

WEEKLY FREQUENCY

The Wilcoxon Signed Ranks Test was used to test the paired difference in change between treatment phases. Little carry-over was noted in the second arm placebo and the placebo data from both arms were included in the analysis.

Paired differences, between treatment arms, in the changes from baseline were found for weekly frequency.

Wilcoxon Signed Ranks Test Statistic Z = -2.1; p= 0.033; Median difference in change = -9 / week 95% C.I. = -19.5, -0.5

The results are illustrated in Table 3.2. Figure 3.3 illustrates the median and 95% C.I. for the primary outcome measure during run-in, and the two treatment arms. The paired difference between active treatment and the other two phases is evident.

	Median	95% Confidence intervals	
		Lower	Upper
Baseline	67.50	54.47	78.00
Active	40.00	36.24	48.00
Placebo	55.00	44.47	65.29
Change from baseline on active treatment	-22.5	-32.76	-10.41
Change from baseline on placebo	-14.00	-20.29	-5.23
Wilcoxon Signed Ranks Test Statistic Z = -2.1; p= 0.033; Median difference in change = -9 / week 95% C.I. = -19.5, -0.5			

Table 4.2
Median of absolute values and their change for weekly frequency

Figure 3

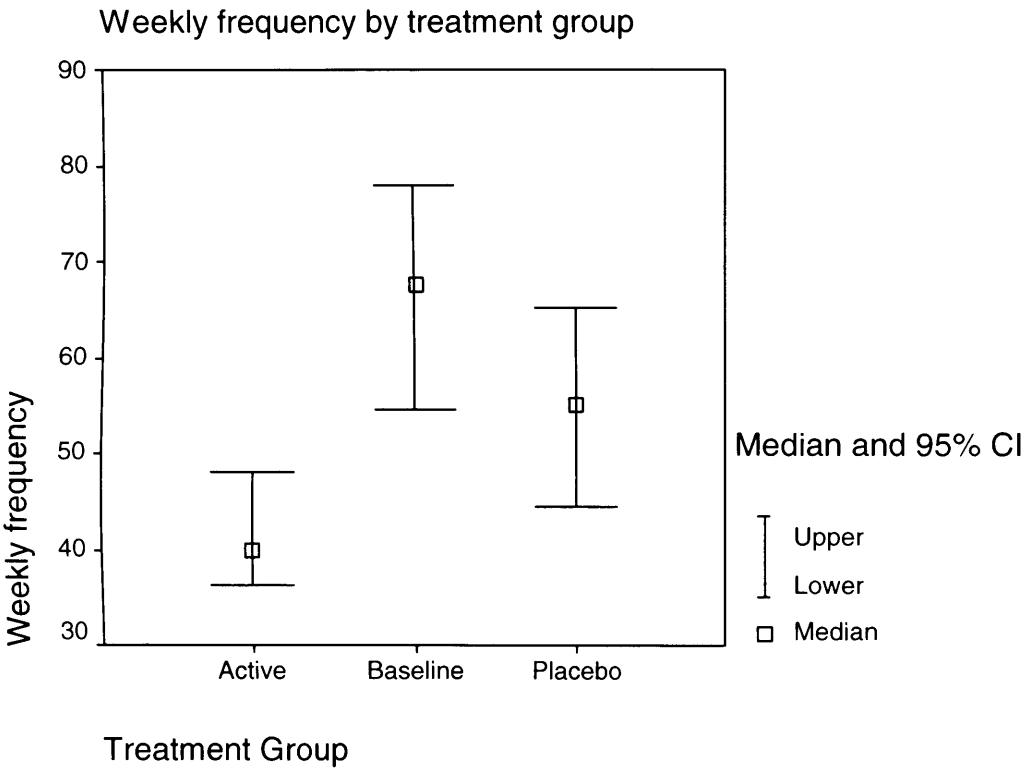


Figure 4.4

The median and 95% C.I. for the weekly frequency during run-in, and the two treatment arms

WEEKLY INCONTINENCE

The Wilcoxon Signed Ranks Test was used to test the paired difference in change between treatment phases. Little carry-over was noted in the second arm placebo and the placebo data from both arms were included in the analysis.

Paired differences, between treatment arms, in the changes from baseline were found for weekly frequency.

Wilcoxon Signed Ranks Test Statistic Z = -2.1; p= 0.033; Median difference in change = -9 / week 95% C.I. = -19.5, -0.5

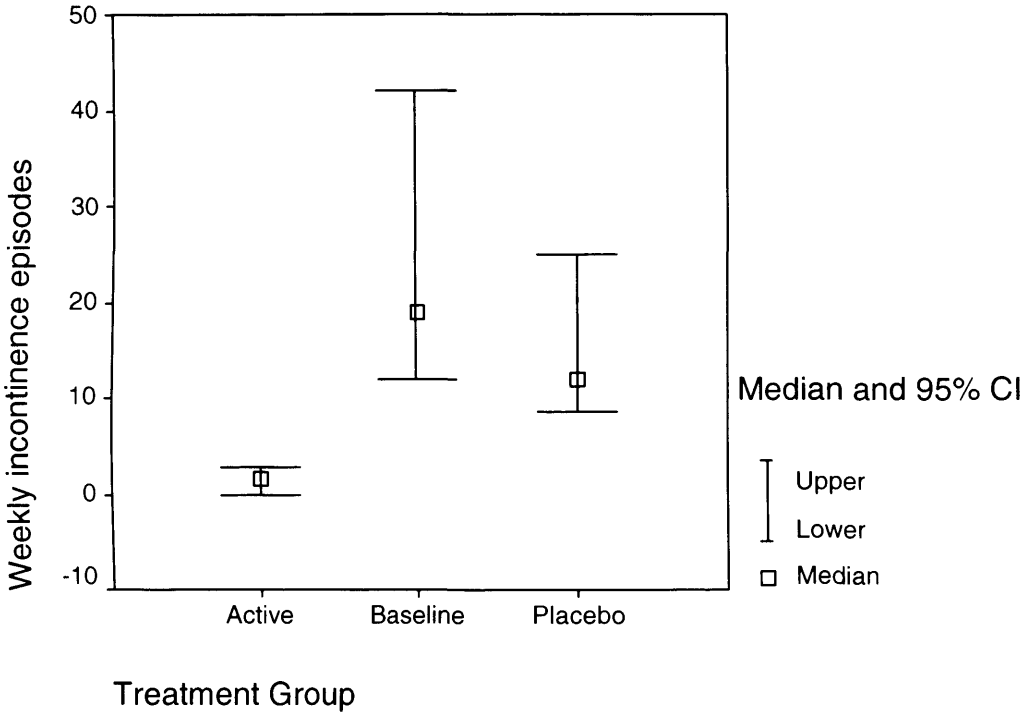
The results are illustrated in Table 3.2. Figure 3.3 illustrates the median and 95% C.I. for the primary outcome measure during run-in, and the two treatment arms. The paired difference between active treatment and the other two phases is evident.

	Median	95% Confidence intervals	
		Lower	Upper
Baseline	19.00	12.00	41.94
Active	1.50	0.00	2.76
Placebo	12.00	8.47	24.82
Change from baseline on active treatment	-17.00	-40.41	-7.70
Change from baseline on placebo	-8.50	-16.53	-2.00
Wilcoxon Signed Ranks Test Statistic Z = -3.3; p= 0.001; Median difference in change = -12 / week 95% C.I. = -24, -5.			

Table 4.3
Median of absolute values and their change for weekly incontinence

Figure 4

Weekly incontinence episodes by treatment group



Treatment Group

Figure 4.5

The median and 95% C.I. for the weekly incontinence during run-in, and the two treatment arms

QUALITY OF LIFE (KINGS HEALTH QUESTIONNAIRE (KHQ))

Nowadays measures of Quality of Life (QOL) are considered to be important additional outcome measures, essential in clinical trials. The International Continence Society (ICS) has recommended that QOL measurements be included in all studies of urinary incontinence as a complement to clinical measures. Patients with detrusor over activity have QOL scores significantly worse than the general population.

The Wilcoxon Signed Ranks Test was used to test the paired difference in change between treatment phases. Little carry-over was noted in the second arm placebo and the placebo data from both arms were included in the analysis.

There were also significant paired differences, between treatment arms, in the change, from baseline, in a number of domains of the KHQ measure of QOL.

Specifically, it was found that active treatment was associated with clinically significant improvements in the impact of the disease on the patient's life, the impact on incontinence, physical and social limitations, sleep/energy disturbance and incontinence severity measure i.e. domains 1, 2, 3, 5, 8 and 9.

Domain		Median	95% Confidence interval		Test Statistic Z	p
			Lower	Upper		
Domain 1 (Present health)	Active	1.00	1.00	1.50	-0.82	0.4
	Placebo	1.50	1.00	2.00		
	Baseline	2.00	1.00	2.00		
Domain 2 (Impact on life)	Active	1.50	1.00	2.00	-2.9	0.004
	Placebo	2.50	2.00	3.00		
	Baseline	3.00	2.50	3.00		
Domain 3 (Incontinence impact)	Active	4.50	2.50	6.50	-2.2	0.03
	Placebo	7.00	5.00	9.50		
	Baseline	9.00	7.50	11.00		
Domain 4 (Role limitations)	Active	2.50	1.00	3.50	-1.8	0.07
	Placebo	3.50	2.50	4.50		
	Baseline	4.00	3.00	4.50		
Domain 5 (Physical/Social limitations)	Active	5.00	3.00	6.50	-2.2	0.03
	Placebo	7.50	5.50	10.00		
	Baseline	9.00	7.50	10.50		
Domain 6 (Personal relationships)	Active	2.00	1.00	4.50	-1.0	0.3
	Placebo	3.50	1.50	5.50		
	Baseline	4.50	3.00	6.50		
Domain 7 (Emotional problems)	Active	5.25	3.00	6.50	-1.6	0.11
	Placebo	7.00	5.00	8.50		
	Baseline	7.50	6.50	9.00		
Domain 8 (Sleep/Energy disturbances)	Active	3.50	2.00	4.50	-2.0	0.04
	Placebo	5.00	3.50	5.50		
	Baseline	5.00	4.50	6.00		
Domain 9 (Incontinence severity measures)	Active	8.50	5.50	10.50	-2.4	0.02
	Placebo	12.00	9.50	14.50		
	Baseline	12.00	10.00	14.00		

Table 4.4
Domains covered by the King's Health Questionnaire. - Median of raw scores with 95% CI and the Wilcoxon Signed Ranks Test statistics

CHAPTER - 5

Chapter 5 SIDE EFFECTS

5.1 BACKGROUND

Botulinum toxin has long been infamous for causing botulism. Some of its earliest and still some of its best clinical descriptions highlight not only paresis but also autonomic dysfunction, especially accommodation problems, reduced sweating, high skin temperature and reduced cerumen production as botulism's cardinal features.

When intramuscular injections were first used to reduce muscular hyperactivity disorders, their effect was strictly local, and no autonomic dysfunction was observed. It was believed that lack of systemic spread was due to a specific and robust BTX-A binding to glycoprotein 'acceptors'.

Later, with the use of higher doses of BTX-A, jitter examinations revealed some systemic motor side effects, residual urine volumes and heart rate variabilities, suggesting some autonomic dysfunction. None of these findings were, however, noticed by the patient nor were they clinically relevant.

There have been quite a few reports of successful usage of BTX A in cases of detrusor overactivity.

None of these studies suggest any adverse effects from these injections apart from a mention of generalised muscle weakness in a couple of patients as a side effect of this treatment [198]

The known side effects of Botulinum toxin: Nausea, vomiting, dry mouth, dysphagia, weakness of the respiratory muscles and paresis have not. [199]

The use of BT-B has been reported to treat successfully a patient with detrusor hyperreflexia secondary to multiple sclerosis. This was followed by an open-label dose-escalation study using BT-B to determine its preliminary efficacy and safety in 15 patients with overactive bladder. No serious side effects were reported, apart from dry mouth in two patients. The dose range used was 2500 IU to 15,000 IU.[111]

There was a recent open controlled study involving 30 consecutive patients in which 24 were treated for Cervical dystonia (11,310 U +/- 2,616U of BTX-B) and 6 were treated for focal Hyperhidrosis (4,000-10,000 U). In 5 of them BTX-A was used additionally for comparison of effectiveness.
In CD, side effects consisted of dryness of mouth (21), accommodation difficulties (7), conjunctival irritation (5), reduced sweating (4), swallowing difficulties (3), heartburn (3), constipation (3), bladder voiding difficulties (2), head instability (1), dryness of nasal mucosa (1) and thrush (1).In Hyperhidrosis, side effects consisted of dryness of mouth (2), accommodation difficulties (4) and conjunctival irritation (1). It was concluded

that autonomic side effects occurred far more often with BTX-B than BTX-A. Their localisation suggested systemic spread.[198]

BTX-B should be applied carefully in patients with autonomic dysfunction, additional anticholinergic treatment and in conditions where anticholinergics are contraindicated.

5.2 SIDE EFFECT PROFILE IN THE CURRENT STUDY

In the current study, a 79-year old man went into urinary retention, as did a 74-year old lady. The urinary retention resolved after six weeks of intermittent self-catheterisation. The man also described constipation, as did a 28-year old woman who additionally experienced a dry mouth. A 62-year old lady noted a dry mouth and general malaise. These side effects, in four patients were self-limiting.

The fact that four of our twenty patients also observed varying side effects including acute urinary retention (2), dry mouth (3), constipation (2) and 'flu-like syndrome' (1) after intravesical injection of 5000 IU of BT-B raises a suspicion that BT-B does seem to have a high affinity for autonomic nerve endings.

The critical dose at which it seems to attack them cannot be decided yet. This is because studies utilising up to 15000 IU have reported little or no side effects while in our study adverse effects were noted at a dose as low as 5000IU.

Currently, there is no standard method for identifying adverse events that occur during a clinical trial. The implications of this lack of consistent ascertainment methods are substantial as comparisons of rates of reported

side effects from 2 or more drugs may not be valid if the methods of collecting adverse events differ. This could impair the ability of patients and physicians to compare the risk– benefit profile of drugs.

Several previous studies have compared the use of open-ended questions and checklists to determine the frequency of reported adverse events in a clinical trial. All of these studies assigned patients to receive both methods of adverse event collection, with the open-ended question being administered first; no randomized comparisons were performed. This method has the potential to bias the participants' response to the checklist because they are initially "primed" by the open-ended question[200, 201].

In a randomized, controlled trial, it was found that a checklist method of identifying adverse events dramatically increased the number of reported events compared with open-ended questions. Although this finding is intuitive, the magnitude of effect has important implications both for the conduct of clinical trials and for assessment of the risk– benefit profile of drugs and other interventions[202].

Since it is common practice for physicians and patients to select drugs and other interventions on the basis of their reported rate of side effects, if different drugs used for the same indication are examined in clinical trials that use different methods of identifying adverse events, then it is not valid to compare the reported rate of side effects.

One could make rational arguments for using a checklist, an open-ended question, or other techniques to identify adverse events in clinical trials. However, use of different methods for collecting data in different clinical trials limits the ability to compare the side effect profile of drugs. The use of the CONSORT (Consolidated Standards of Reporting Trials) statement has helped to make clinical trials more uniform and comparable[203].

CHAPTER - 6

Chapter 6 HISTOLOGY

6.1 INTRODUCTION AND BACKGROUND

Botulinum toxin selectively blocks the release of acetylcholine from nerve endings by inhibiting the exocytosis of presynaptic acetylcholine vesicles. Ultrastructural studies of the neurogenic overactive detrusor due to spinal cord injury, meningomyelocele and brain disorders, that have been treated with botulinum toxin type A, have demonstrated changes in the intrinsic innervation including a widespread axonal degeneration and a limited axonal regeneration combined with schwann cell activation independent of the duration of the neurogenic disorder[204].

Myogenic changes included the presence of different extents of muscle degeneration, a reduction of intermediate cell junctions of muscle cells and a dominance of intimate cell appositions with much narrower junctional gaps forming chains >= 5 muscle cells[205-207].

Local changes (viz. inflammation, fibrosis, necrosis etc.) after the injection of Botulinum toxin B into the overactive detrusor, however, have not been looked at, till date. This is apposite because the agent is an imunogenic peptide that is known to induce an antibody response. If this treatment were to induce a local acute or chronic inflammatory reaction, it would be a very significant matter.

6.2 METHODS

20 patients with refractory detrusor overactivity were recruited for this randomised, double bind, placebo controlled, cross over trial.
An attempt was made to obtain bladder biopsies from all the patients on each of their visits to the day surgery unit.

Since the procedure was done under sedo analgesia, not all the attempts to biopsy the bladder were successful. Some of the patients found the cold cup biopsies quite uncomfortable and the procedure had to be abandoned in these cases.

In all 26 biopsies were obtained from 20 patients and were divided into three groups:
Group I: Biopsies obtained from the normal detrusor (before any injection)
Group II: Biopsies after 6 weeks of intradetrusor injection of placebo (Normal saline)
Group III: Biopsies after 6 weeks of intradetrusor injection of botulinum toxin B

There numbers were as follows:
Group I: 13
Group II: 8
Group III: 5

These biopsies were prepared using the standard methods of fixation, mounting and staining with Haematoxylin and Eosin (H & E). The resulting slides were then evaluated for local changes by two examiners (Maneesh Ghei and Dr Debbie Hopster) both blinded to the clinical / urodynamic data.

6.3 PRACTICAL PROCEDURES

TISSUE PROCESSING

Once the tissue was fixed in formalin, it was processed into into thin microscopic sections. The tissues embedded in paraffin wax, which is similar in density to tissue. The blocks thus prepared were sectioned using a microtome into slithers of 6-8 microns in depth.

The main steps in this process were dehydration and clearing.

Wet fixed tissues (in aqueous solutions) cannot be directly infiltrated with paraffin. First, the water from the tissues was removed by dehydration. This was usually done with a series of alcohols, 70% to 100%. Other dehydrants can be used, but have major disadvantages.

The next step was "clearing" and consisted of removing the dehydrant with a substance that was miscible with the embedding medium (paraffin). Xylene was used for this purpose.

Finally, the tissues were infiltrated with the embedding agent, paraffin.

This "embedding" process is very important, because the tissues must be aligned, or oriented, properly in the block of paraffin.

Once the tissues were embedded, they were cut into sections that could be placed on a slide. This was done with a microtome. The microtome is nothing more than a knife with a mechanism for advancing a paraffin block standard distances across it.

Once sections were cut, they were floated on a warm water bath that helped remove wrinkles. Then they were picked up on a glass microscopic slide. The embedding process was reversed in order to get the paraffin wax out of the tissue and allow water soluble dyes to penetrate the sections. Therefore, before any staining was done, the slides were "deparaffinized" by running them through xylenes (or substitutes) to alcohols to water. This was done since there are no stains that can be used on tissues containing paraffin.

H and E staining

This is the most routinely used method of staining. All the other stains are called special stains.

Haematoxylin is the oxidized product of the logwood tree known as haematin. Since this tree is very rare nowadays, most haematin is of the synthetic variety. In order to use it as a stain it must be "ripened" or oxidized. This can be done naturally by putting the haematin solution on the shelf and waiting several months, or by buying commercially ripened haematoxylin or by putting ripening agents in the haematin solution.

Haematoxylin will not directly stain tissues, but needs a "mordant" or link to the tissues. This is provided by a metal cation such as iron, aluminium, or tungsten. The variety of haematoxylins available for use is based partially on choice of metal ion used. They vary in intensity or hue. Haematoxylin, being a basic dye, has an affinity for the nucleic acids of the cell nucleus.

Haematoxylin stains are either "regressive" or "progressive". With a regressive stain, the slides are left in the solution for a set period of time and then taken back through a solution such as acid-alcohol that removes part of the stain. This method works best for large batches of slides to be stained and

is more predictable on a day to day basis. With a progressive stain the slide is dipped in the haematoxylin until the desired intensity of staining is achieved, such as with a frozen section. This is simple for a single slide, but lends itself poorly to batch processing.

Eosin is an acidic dye with an affinity for cytoplasmic components of the cell. There are a variety of eosins that can be synthesized for use, varying in their hue, but they all work about the same. Eosin is much more forgiving than haematoxylin and is less of a problem in the lab. About the only problem one sees is overstaining, especially with decalcified tissues.

6.4 RESULTS

Of the 26 biopsies obtained, one was inadequate and one of the pots did not contain a specimen.

Hence 24 biopsies were examined by two examiners, one a pathologist, who were blinded to the clinical data.

Not all the biopsies included muscularis propria. Their content has been described in table 5.1

The mounted preparations were scrutinised for the following changes:

- Acute inflammation
- Chronic inflammation
- Fibrosis
- Necrosis and
- Vascularity

A mixture of all these patterns was observed in all the biopsies and this has been tabulated in table 6.2 and figs 6.1-6.5.

No pattern dominated the picture significantly in any of the biopsies.

It would be wrong to propose that the chronic inflammation observed in these biopsies was a result of the injections since it was seen in the pre injection biopsies as well. It may be that these findings suggest that cystitis is associated with overactive bladder.

It has been suggested that the link between inflammation and the Painful Bladder Syndrome / Interstitial Cystitis symptoms of frequency, urgency, and

pain may lie in the bladder's neuroplastic response to inflammation. Irrespective of the type of inflammatory stimulus present, the bladder has been shown to respond with an increase in cytokine production (mainly Nerve growth factor (NGF) production, as well as with morphologic changes in sensory neurons that innervate the bladder.

Basic science studies have shown that various insults to the bladder (eg, mechanical, chemical, immune) lead to increased NGF production and morphologic changes (ie, neuroplasticity) in sensory and motor neurons. Different insults, including a bladder infection, theoretically could result in a common neuroinflammatory response that potentially could lead to persistent symptoms, even when the initial inflammatory stimulus subsides[208].

Further support of the role of inflammation in overactive bladder and PBS/IC was demonstrated by Jang et al. In a rodent model of overactive bladder, they demonstrated that intravesical instillation of nonsteroidal anti-inflammatory drugs can alter the expression of inflammatory modulators and cytokines in bladder tissue. Cyclooxygenase- 2 inhibitors reduced the expression of inducible nitric oxide synthase and NGF in treated rats. The effect was also seen as improvements in parameters of detrusor contraction when compared with rats with untreated overactive bladder[209].

Furthermore, Saito et al. tested the use of the anti-inflammatory drug loxoprofen on lower urinary tract symptoms in men and women with BPH

and/or overactive bladder. Specifically, there was a reduction of nocturia in these patients after 1 week of treatment[210].

In an experimental model of cystitis, Gonzalez et al. found that lipopolysaccharide from the bacteria *Escherichia coli* caused an increase in NGF and substance P. In addition, treatment with RDP58, a synthetic peptide that inhibits early signal transduction pathways for the expression of inflammatory cytokines, decreased NGF and substance P production along with histopathologic inflammation. Therefore, by decreasing the production of inflammatory and neurotrophic factors, RDP58 modulates the neuroplastic response to inflammation and may play a role in treating inflammatory bladder disorders[211].

Thus the complex association between inflammatory cells, cytokines, and the nervous system may be responsible for the pathogenesis and progression of overactive bladder, and PBS/IC symptoms. Inflammatory cells and their cytokines, found in tissue or in secretions, may act as an extension of the peripheral nervous system in the bladder's immunosensory loop, mediating the symptoms of all these syndromes.

One of the specimens examined post-botulinum toxin B (6 weeks after the injection) did show necrosis in the muscularis propria. There were no grounds for judging whether this resulted from the injection or it was a process of the overactive bladder.

This phenomenon was not observed in any of the other biopsies obtained post-botulinum toxin B injections.

The histology results from our study concur with the general experience that Botulinum toxin does not cause fibrosis of the muscles local to the site of injection when it is used for other indications. The number of biopsies in the present study was small and we studied the effect of the injections after six weeks only.

We do not know whether the smooth muscles will react differently when repeated injections are used and at a higher dosage. Studies with larger numbers and longer follow ups would be required to ascertain definitively, whether fibrosis or other derogatory changes occur to the smooth muscle after Botulinum toxin injections.

BIOPSY	EPITHELIUM	MUSCULARIS MUCOSA	MUSCULARIS PROPRIA
A	+, D, N	+, N	-
B	+, D	+	+, N
C	+, N	-	-
D	+, N	-	+
E	+, D, N	+	-
F	+, N	+	-
G	+, D, N	+	-
H	+, N	+	-
I	+, D	-	+
J	+, D, N	+	-
K*	+, N	-	-
L	+, N	-	-
M	+, N	+	+
N	D	+	-
O	+, D, N	+	-
P	+	+	+
Q	+	+	-
R	+	-	+
S	+, D, N	+	+
T	+	+	-
U	+, D, N	+	-
V	+, D	-	-
W**			
X	+	-	+
Y	+, D, N	-	-
Z	+	-	+

D: *Denuded* N: *Normal* *: *Suboptimal* **: *No specimen*

Table 6.1
Biopsies containing epithelium, lamina propria and muscularis propria

SPECIAL NOTE

THIS ITEM IS BOUND IN SUCH A MANNER AND WHILE EVERY EFFORT HAS BEEN MADE TO REPRODUCE THE CENTRES, FORCE WOULD RESULT IN DAMAGE

BIOPSY	ACUTE INFLAMMATION	CHRONIC INFLAMMATION	FIBROSIS	VASCULARITY	NECROSIS
A	-	+	-	P	-
B	-	+	-	NP	-
C	-	+	-	NP	-
D	-	+	-	NP	+
E	-	+	-	P	-
F	-	+	-	P	-
G	-	+	-	P	-
H	-	+	-	P	-
I	-	+	-	P	-
J	-	+	-	P	-
K	*SUBOPTIMAL SPECIMEN*				
L	-	+	-	P	-
M	-	+	++	P	-
N	-	+	-	NP	-
O	-	++	-	P	-
P	-	+	+	P	-
Q	+	+++	-	P	-
R	-	+++	-	P	-
S	-	+	-	P	-
T	-	+	-	P	-
U	-	++	-	P	-
V	-	+	-	NP	-
W	*NO SPECIMEN*				
X	-	+	++	P	-
Y	-	+	-	NP	-
Z	-	+	+	P	-

P: *Prominent* NP: *Not prominent*

Table 6.2
Biopsies with evidence of acute and chronic inflammation, fibrosis and necrosis

CODE	BIOPSY TYPE
A	NORMAL
B	POST NORMAL SALINE
C	NORMAL
D	POST BOTULINUM TOXIN B
E	NORMAL
F	POST NORMAL SALINE
G	NORMAL
H	POST BOTULINUM TOXIN B
I	NORMAL
J	POST NORMAL SALINE
K	NORMAL
L	POST NORMAL SALINE
M	POST NORMAL SALINE
N	POST BOTULINUM TOXIN B
O	NORMAL
P	POST NORMAL SALINE
Q	NORMAL
R	POST BOTULINUM TOXIN B
S	NORMAL
T	POST NORMAL SALINE
U	POST NORMAL SALINE
V	NORMAL
W	NORMAL
X	NORMAL
Y	NORMAL
Z	POST BOTULINUM TOXIN B

Table 6.3
Key for Biopsy codes

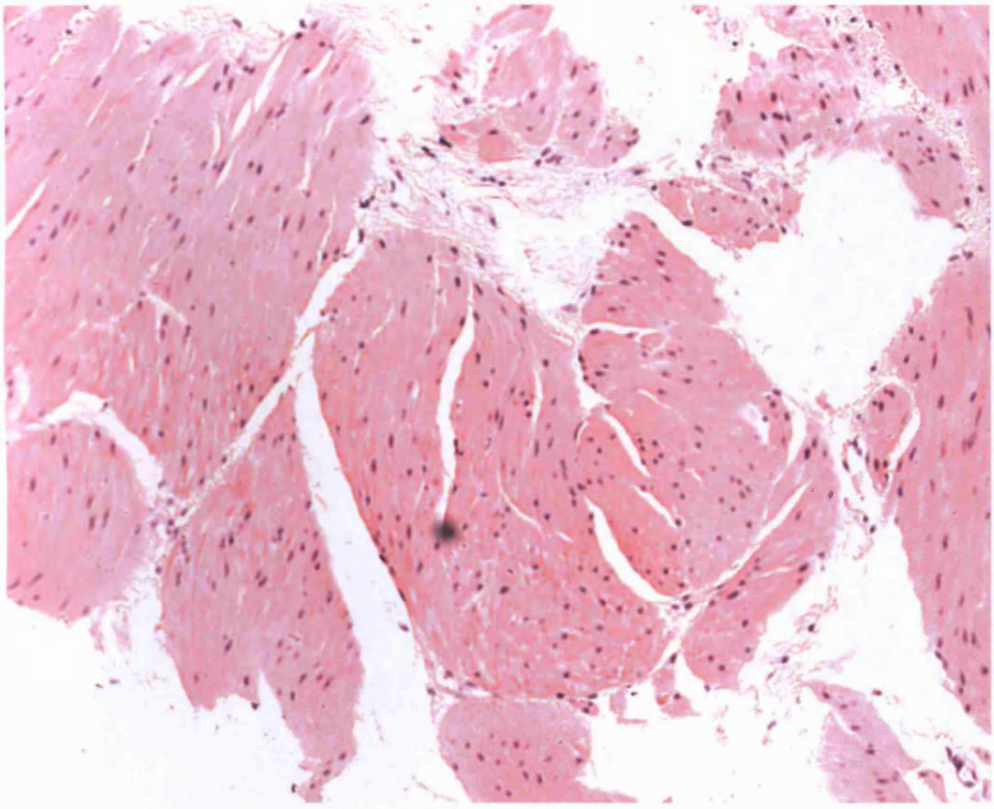


Figure 6.1
Normal Detrusor

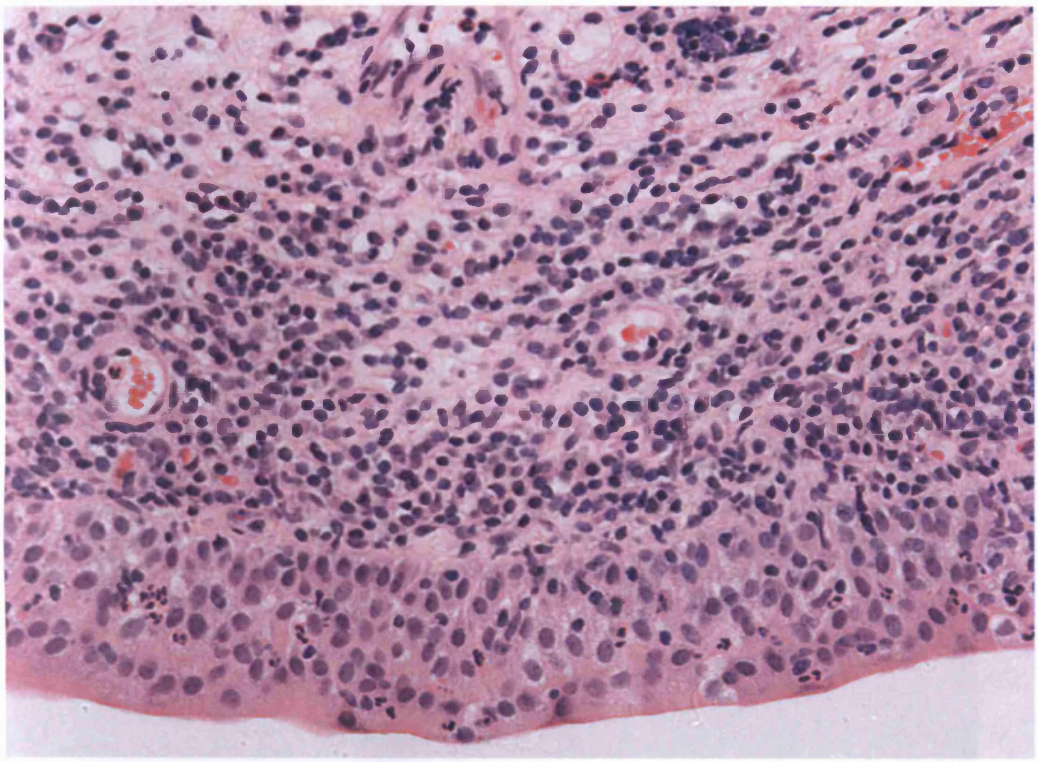


Figure 6.2
Detrusor showing
aggregates of neutrophils
in Acute inflammation

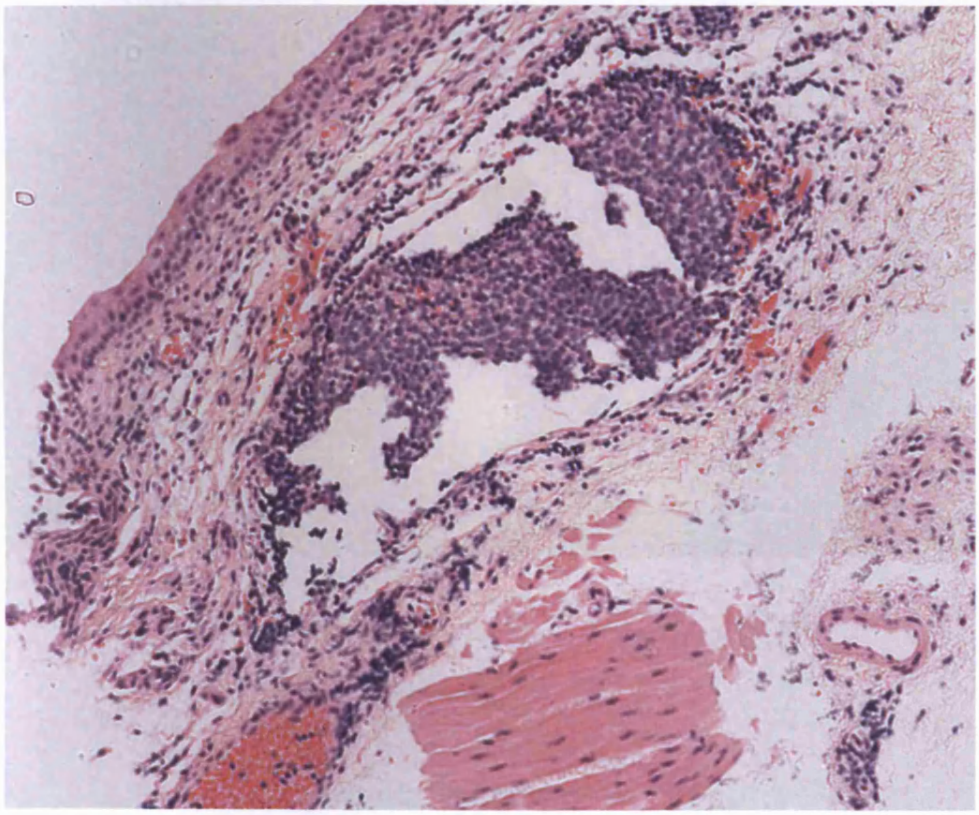


Figure 6.3
Detrusor showing
aggregates of lymphocytes
(lymphoid follicle) in
Chronic inflammation

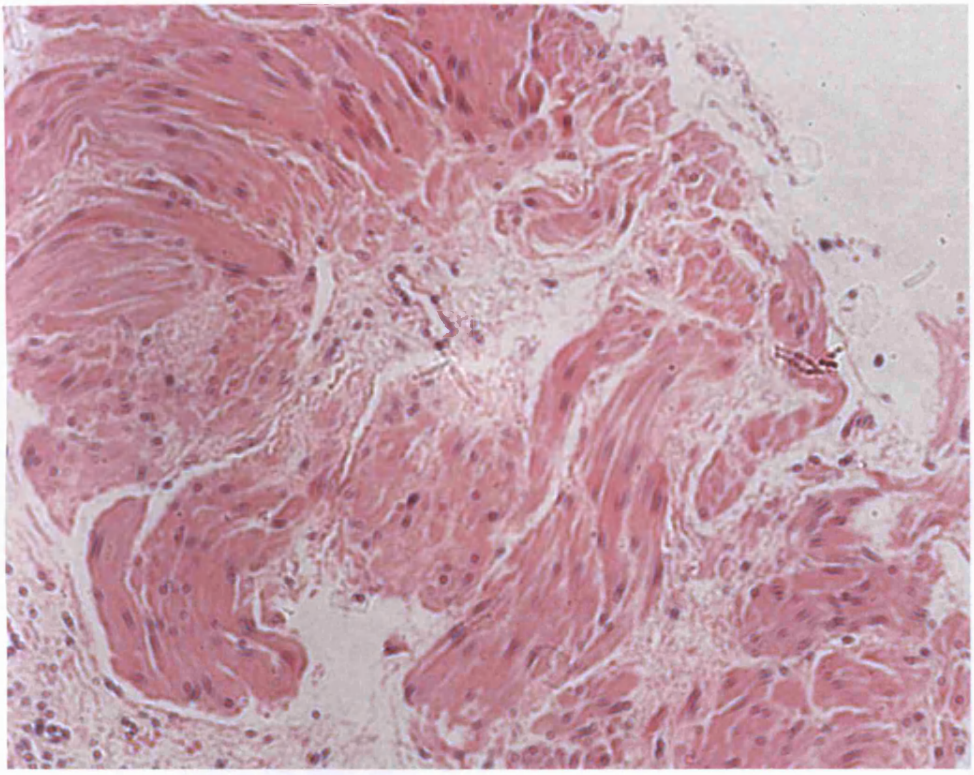


Figure 6.4
Detrusor with fibrosis

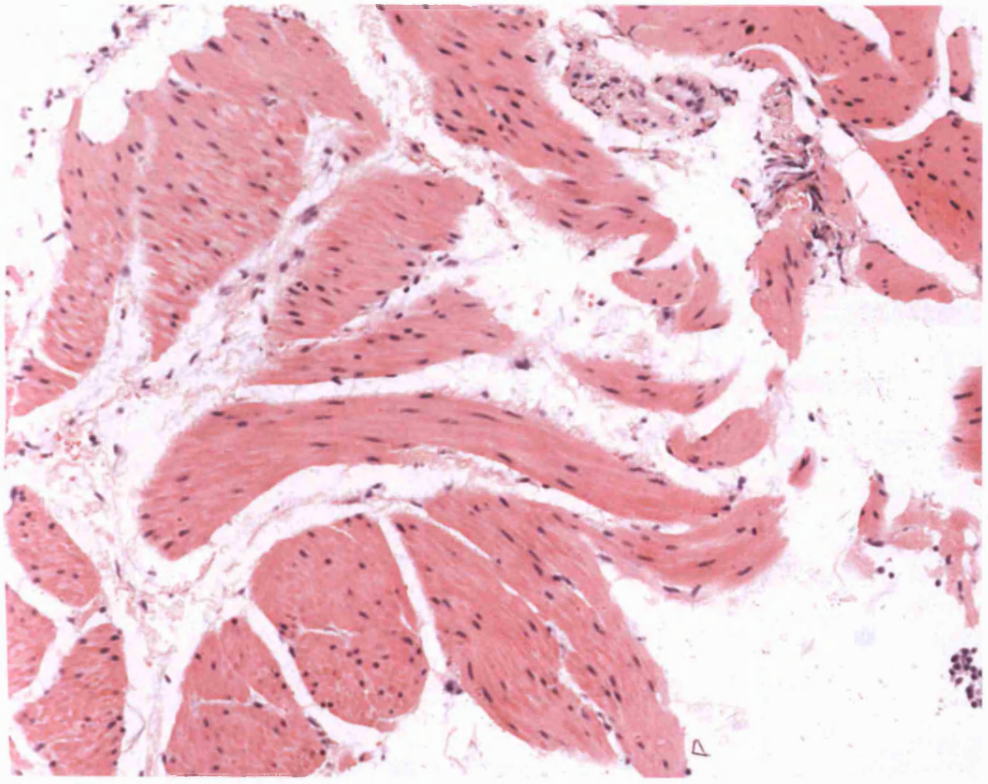


Figure 6.5
Detrusor with necrosis

CHAPTER - 7

Chapter 7 DISCUSSION

7.1 PRESENT STUDY

This randomised double-blind, placebo controlled crossover study has furnished evidence in favour of the efficacy of Botulinum Toxin B in the treatment of recalcitrant overactive bladder symptoms. The study was properly powered and the evidence from the primary outcome measure was reflected by the secondary measures including those assessing Quality of Life.

The experiment illustrates the utility of a cross-over design. This has not been favoured for studies of urinary incontinence because of speculative beliefs about the influence of carry-over effects confounding the treatment response. Unlike an oral medication, with a short half-life, BTX-B showed measurable effects up to six weeks and there is evidence of a carry-over into the placebo arm. Nevertheless, this did not prohibit a between phase analysis using all of the placebo data. As this study illustrates, the design permits a much smaller sample size with the associated reduction in costs and logistical difficulties. Fewer patients are inconvenienced and the important efficacy data can be provided in a much shorter time.

The data were analysed on an intention-to-treat basis. Two patients withdrew after receiving active drug. The non-parametric methods used in the analysis should minimise the effect of their data.

This center bought the test drug independently on the open market. The placebo and active components were prepared by the hospital pharmacy. The study was effected independently of the manufacturer [212].This took place before the European Clinical Trials Directive 2001/20/EC came into effect on 1st May 2004, whilst it is still possible to accomplish such a study, now it would be more difficult and expensive. The host Trust would have to agree to "Sponsor Status" and the indemnity costs associated with that could be high.

7.2 LIMITATIONS AND CRITICISMS OF THE STUDY

Possible error could have been introduced through underestimate of carry-over effect but this would have reduced apparent efficacy. The dose of drug was based on a pilot study by Dykstra DD (2003) on 15 patients which used dose escalation. It may be that the dose of 5,000 IU was not optimal. Enquiries were made about side effects without leading questions so these may have been underestimated.

This treatment was not without side effects. Urinary retention, requiring intermittent catheterisation was a theoretical risk, about which all patients were warned. The data from this experiment mean that is very important that patients understand when making judgments on the balance of gain. Antimuscarinic side-effects, excluding urinary retention, have been noted when BTX has been used to treat dystonias [199].

This study of 5,000 IU of BTX-B shows an effect lasting about six weeks. BTX-A has been used more widely to treat the overactive bladder, detrusor hyperreflexia and sphincter dyssynergia. Side effects of BTX-A including, nausea, vomiting, dry mouth, dysphagia, weakness of respiratory muscles and paralysis have been described following its use for various dystonias and other indications. As regards patients treated locally for lower urinary tract dysfunction, in the literature there is a single case report of generalised

muscle weakness [198]. Reports of 200 to 300 IU of BTX-A injected for detrusor hyperreflexia, in spinal cord injured patients describe a protracted effect lasting at least 24 weeks [66].

A longer duration of action confers benefit. If the incidence of side effects proves similar, BTX-B because of its more limited action duration, might find a role as a means of testing tolerance to BTX treatment. Otherwise, it will presumably be limited to use in patients who have experienced tachyphylaxis with BTX-A. It is well-recognised that BTX is immunoreactive and stimulates a polyclonal-antibody response to its peptide structure.

Many of the antibodies do not influence efficacy but with time there may be degradation of effect, depending on the antibody binding site, so that the drug loses its influence, as has occurred with cervical dystonias and recently with detrusor hyperreflexia [109, 213-215]. It has been found that there is very little cross-reactivity of antibodies between the different BTX molecules so that BTX-B should prove useful following attenuation of the primary response to BTX-A.

7.3 FUTURE STUDIES

Many questions are raised by these studies. Future studies should include looking more closely into subgroups (neuropaths and non-neuropaths), which the present study was not able to.

It would be useful to study a larger group of patients with refractory detrusor overactivity, followed over a longer period of time with repeated injections.

A higher dose of Botulinum toxin B could be tried and a formal dose-titiration study would be useful in guiding therapeutic decisions.

The study showed the potential affinity of the toxin for autonomic nerves and I think this should be investigated further by means of properly powered trials. Peripheral autonomic effects could present a very significant hazard. In Guillaune Barre syndrome, the great fear and the cause of mortality is autonomic failure.

It would also be useful to look into developing specific strains of botulinum toxin (by means of genetic engineering) which would increase the longevity of its effects, decrease or minimise the side effects and hence cut down the need for repeated injections.

It would be helpful to generate a strain which encourages minimal antibody production.

In recent years there has been increasing evidence that BTX-A might also have analgesic properties[216]. Initially, this was thought to be due to relief of muscle spasm. However, botulinum has been shown to reduce peripheral sensitization by inhibiting the release of several neuronal signaling markers,

including glutamate and substance P, and reducing c-fos gene expression. It may affect the sensory feedback loop to the central nervous system by decreased input from the muscle tissue, possibly by inhibiting acetylcholine release from gamma motor neurons innervating intrafusal fibers of the muscle spindle [217]. BTX-A has been used effectively for years in different conditions with muscular hypercontractions. Intradetrusor BTX administration blocks the acetic acid-induced calcitonin gene-related peptide (CGRP) release from afferent nerve terminals in the bladder mucosal layer in rats. In an animal model of bladder permeability barrier disruption, intradetrusor BTX-A minimized bladder irritability and restored afferent neural responses to baseline levels [218]. These results support clinical trials of BTX-A for the treatment of PBS/IC and other types of visceral pain [219].

A multi-institutional case series using Botox or Dysport intradetrusor injections in 13 patients with refractory PBS/IC reported improvement in 9 patients. Improvements in symptoms lasted a mean of 3.72 months (range, 1 to 8 months). No systemic complications were observed, although 2 patients had a diminished flow with some need to strain to void [220]. Rackley and colleagues at the Cleveland Clinic reported no change in objective or subjective outcome measures in a series of 10 PBS/IC patients in whom the trigone was spared in the injection technique [221]. At this time, Botox can be recommended for PBS/IC use only in the context of carefully controlled clinical trials.

The clinical method adopted for this study deserves some comment. For many years now, the standard approach for testing the efficacy of treatments for the overactive bladder has been the parallel group study. It would seem

that this is based on the assumption that a carry-over effect following the first limb suing active treatment would result in a failure of discriminating power. It is interesting to note that the available literature does not describe the length of drug effect, nor a time series describing the change in symptoms following the cessation of an antimuscarinic agent. Some believe that the measurement frame would be compromised by the more protracted influence of a bladder training effect. A recent J Urol publication challenges the effect of bladder retraining on urinary incontinence. [222]

In this study we worked on the assumption, based on previous work, that the carry-over effect would be small so as not to cloud a therapeutic response. This proved to be the case. If there were a training effect, both pathways would be similarly affected. If there were a drug carry over effect it would be likely to be markedly attenuated.

In crossover studies all patients serve as own controls and error variance is reduced thus reducing the sample size needed; All patients receive treatment (at least some of the time). Statistical tests assuming randomisation can be used validly. Blinding can be maintained. Thus the design experiments with the same power to influence practice but with a fraction of the cost and a sample size that makes a single-centre operation feasible.

The disadvantages are that all subjects receive placebo or alternative treatment at some point. The washout period could be lengthy or unknown,

although this study illustrates how this can be overestimated. It cannot be used for treatments with permanent effects.

Since clinical trials of treatments for the overactive bladder were first attempted, the sample sizes used have been increasing. Two recent controlled trials randomised 911 and 1081 patients, respectively in order to detect a between groups differences of 1 micturition episode per 24 hours[223,224]. However, in 1999 a similar study randomised 316 patients in order to effect the same analysis [225]. Inflation in recruitment is far from ideal since it proves time-consuming, expensive, demanding of logistical effort and inconvenient for the patients. It is not reasonable to randomise large numbers of patients to placebo if the hypothesis could be tested as well with a smaller sample.

7.4 CONCLUSIONS

This study provides evidence of efficacy of Botulinum Toxin B in the treatment of the overactive bladder. The data suggest that this formulation and dose has a rapid onset of action (24 to 48 hours) but short duration, about six weeks.

However, it is not without any side effects. There was evidence of dry mouth, constipation, urinary retention and 'flu-like' syndrome which suggested the affinity of the toxin for autonomic nerves and its potential systemic absorption.

The experiment illustrates the utility of a cross-over design. This has not been favoured for studies of urinary incontinence because of speculative beliefs about the influence of carry-over effects confounding the treatment response.

Unlike an oral medication, with a short half-life, BTX-B showed measurable effects up to six weeks and there is evidence of a carry-over into the placebo arm.

Botulinum toxin B, because of its more limited action duration, might find a role as a means of testing tolerance to Botulinum toxin class, although this hypothesis would require testing. Otherwise, it will presumably be limited to use in patients who have experienced tachyphylaxis with Botulinum toxin A.

CHAPTER - 8

Chapter 8 References

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SPECIAL NOTE

THIS ITEM IS BOUND IN SUCH A MANNER AND WHILE EVERY EFFORT HAS BEEN MADE TO REPRODUCE THE CENTRES, FORC WOULD RESULT IN DAMAGE

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APPENDIX 1

PATIENT INFORMATION SHEET

PATIENT INFORMATION SHEET

EFFECTS OF BOTULINUM TOXIN ON UNSTABLE BLADDERS

We are inviting you to take part in this research for the treatment of your unstable bladder. Before you decide, it is important that you understand why the research is being done and what it will involve and any risks that there may be in taking part. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the research?

To find a possible cure for the unstable bladders so reducing the discomfort caused by urgency and incontinence and the repeated visits to the toilet associated with it.

Why you?

You have been asked to participate because you have an unstable bladder and have tried other forms of treatment without any success. A total of twenty patients will be invited to participate.

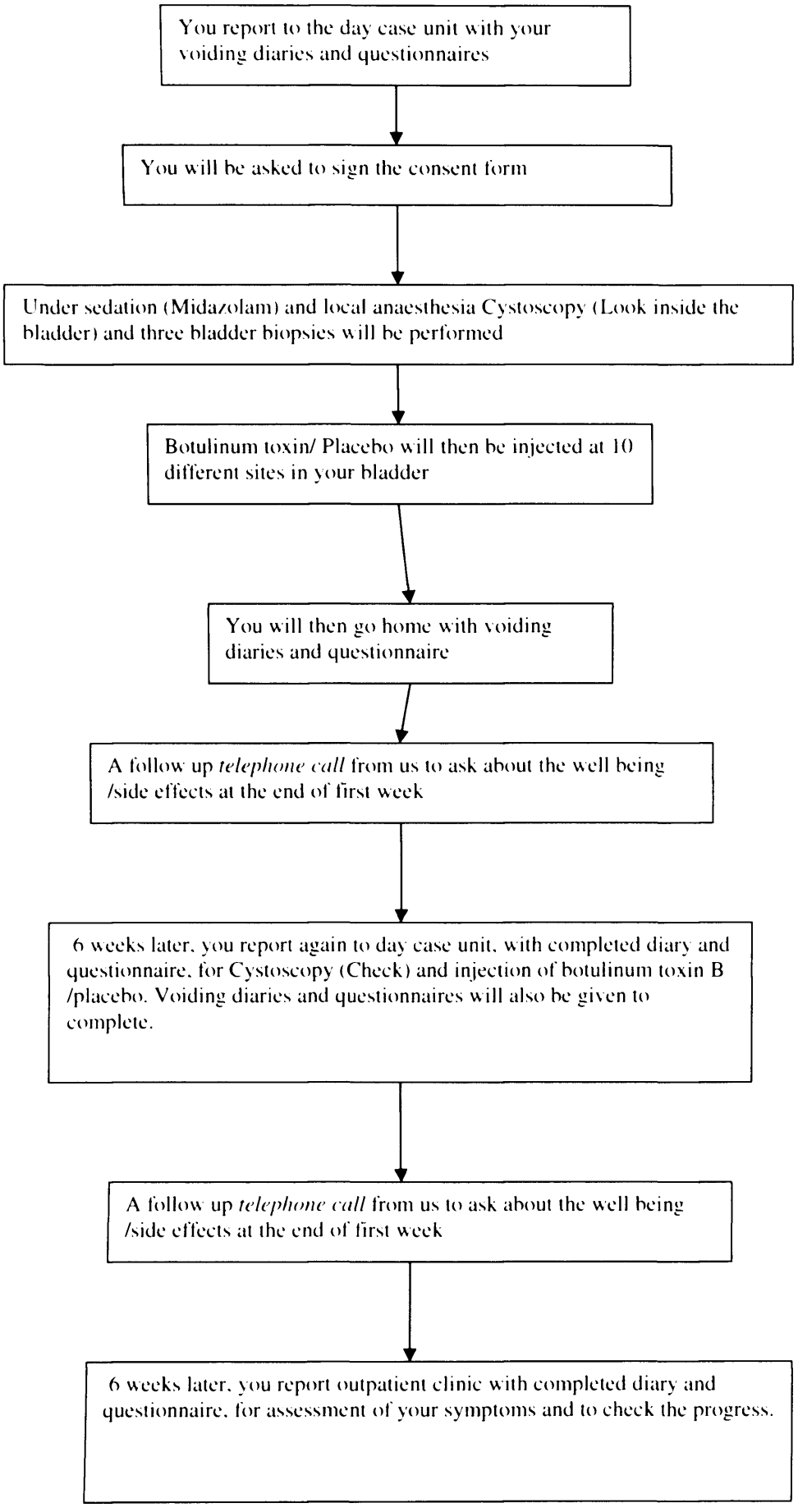
Are you obliged to take part?

|No.It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw from the study at anytime and without giving any reason. This will not affect the standard of care you receive.

SPECIAL NOTE

THIS ITEM IS BOUND IN SUCH A MANNER AND WHILE EVERY EFFORT HAS BEEN MADE TO REPRODUCE THE CENTRES, FORCE WOULD RESULT IN DAMAGE

hat will happen to you if you take part?



Effects on your daily life?

- ✓ There are no life style restrictions associated with the study.
- ✓ You can drive as normal the next day.
- ✓ There are no dietary restrictions.
- ✓ You can continue to take your regular medications.
- ✓ You need to drink plenty of fluids over the next couple of days of the procedure to cleacr the small amount of blood you might notice in your urine.

What is the drug being tested?

Botulinum toxin is a neurotoxic drug safely used for the past 20 years to control various muscular disorders of a neurological origin. It is licensed for use in treating muscular spasms(in the neck and shoulders), excessive sweating of the armpits and treatment of spasticity, visual squints and some digestive tract problems. Botulinum toxin is not yet licensed for use in the bladder, however, in recent years physicians in both Europe and America have reported its successful use in treatment of bladder over activity, which is one of the commonest causes of urinary incontinence.

What are the possible benefits of taking part?

We hope that the treatment will help you. However, this can not be guaranteed. The information we get from this study may help us treat future patients with unstable bladders better.

What are the side effects of the treatment?

There are no known major side effects of the treatment as yet although a very few section of patients have experienced flu-like symptoms, dry mouth and drowsiness (less than 1%). No cases of paralysis of muscles outside the bladder have been reported.
However, after the procedure you may experience some blood (slight amount) in your waterworks for a couple of days which will clear up if you drink plenty of fluids for that time.
There is also slight risk of going in to retention of urine which may require self catheterisation for some time, however no cases of this side effect have been reported so far from similar studies.

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/drug that is being studied. If this happens, your research doctor will tell you about it and discuss with you whether you want to

continue in the study. If you decide to withdraw your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/She will explain the reasons and arrange for care to continue.

What happens when the research study stops?

This treatment will not be available after this research study stops. Many of other centres have been showing interest in this study and it might just be possible for the treatment to be available there.

What will happen to the results of my study?

The results of your study are likely to be published. In case you wish to get a copy of the published results, a copy may be requested for by your research doctor conducting the study.

What if something goes wrong?

We will take every care in the course of this trial. If through our negligence any harm results you will be compensated. However a claim may need to be pursued through legal action. The NHS Trusts are not permitted to carry indemnity for non-negligent (no fault) harm.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the study will be kept strictly confidential. Any information about you which leaves the hospital/surgery will have your name and addresses removed so that you cannot be recognised from it.

Who is organising and funding the study?

The study will be conducted at the Whittington hospital. The research is not being sponsored by any drug company, however the drug so required will be provided by the manufacturers of the same ***(ELAN PHARMA).***

Who has reviewed the study?

This studied has been reviewed by the ***Whittington Hospital Research Ethics Committee.***

Contact for further information?

The following doctors will be involved in the study:
Mr. Ron Miller : Consultant Urologist, Whittington hospital
Prof. James Malone-Lee: Consultant, Department of Medicine, Whittington hospital
Dr. Maneesh Ghei : Research registrar, Whittington hospital

You may contact them at any time through the **hospital switchboard 02072723070**

You will be given a copy of this Information sheet and a signed consent form to keep

APPENDIX 2

CONSENT FORM

CONSENT FORM

(**version** 2 **dated** 23rd June 2003)

Title of Project:

Effects of Botulinum Toxin on Unstable Bladders

Name of Researcher:
Prof. James Malone-Lee (Consultant, Dept. of Medicine)
Maneesh Ghei (Research Registrar)
Ron Miller (Consultant Urologist) &
Senthil Nathan (Consultant Urologist)

Please initial box

1. I confirm that I have read and understood the information sheet dated 23rd June 2003 (Version...2) or the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from Whittington Hospital or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

5. I also understand that the biopsies obtained from my bladder will be used for histopathological examination and other analysis and I give my permission for the same. These tissues may be treated as donation from me and I have been told that there will be no financial remunerations for supplying the tissue to other organisations or individuals. ☐

4. I agree to take part in the above study. ☐

Name of Patient	Date	Signature
Researcher	Date	Signature